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(54) METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS

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Continuation-in-part of application No. 09/663,968, filed on Sep. 19, 2000.

(60) Provisional application No. 60/217,658, filed on Jul. 10, 2000. Provisional application No. 60/159,176, filed on Oct. 13, 1999. Provisional application No. 60/217,251, filed on Jul. 10, 2000.

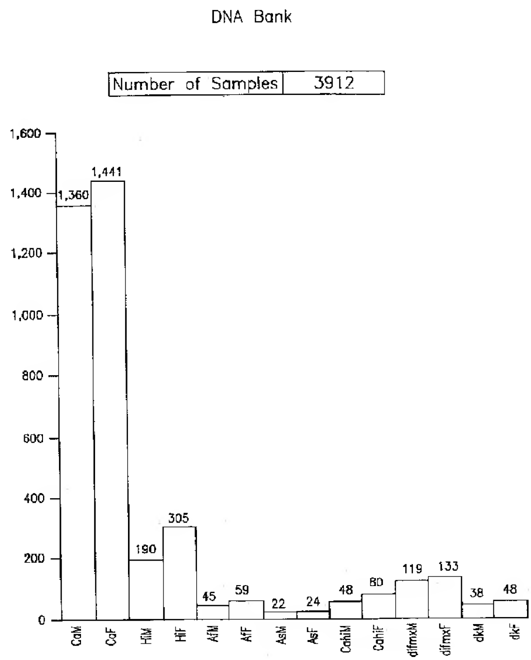
Publication Classification

(51) Int. Cl.[7] **C12Q 1/68**; G06F 19/00; G01N 33/48; G01N 33/50

(52) U.S. Cl. **435/6**; 702/20

(57) **ABSTRACT**

Processes and methods for creating a database of genomic samples from healthy human donors, methods that use the database to identify and correlate polymorphic genetic markers and other markers with diseases and conditions are provided.



DNA Bank

Number of Samples	3912

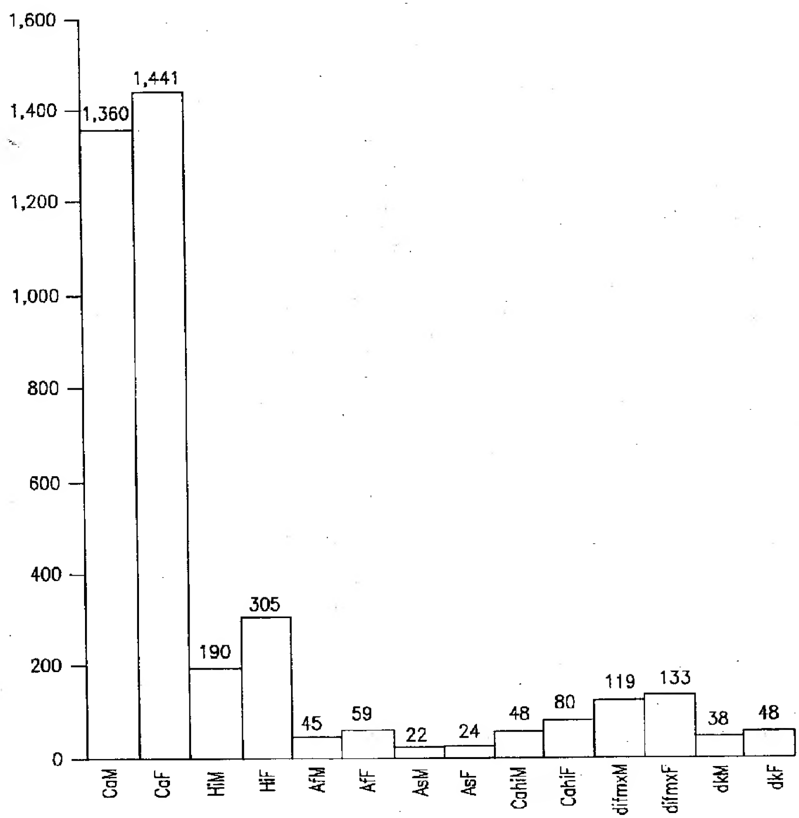


FIG. 1A

Caucasians

Number of Samples	2801

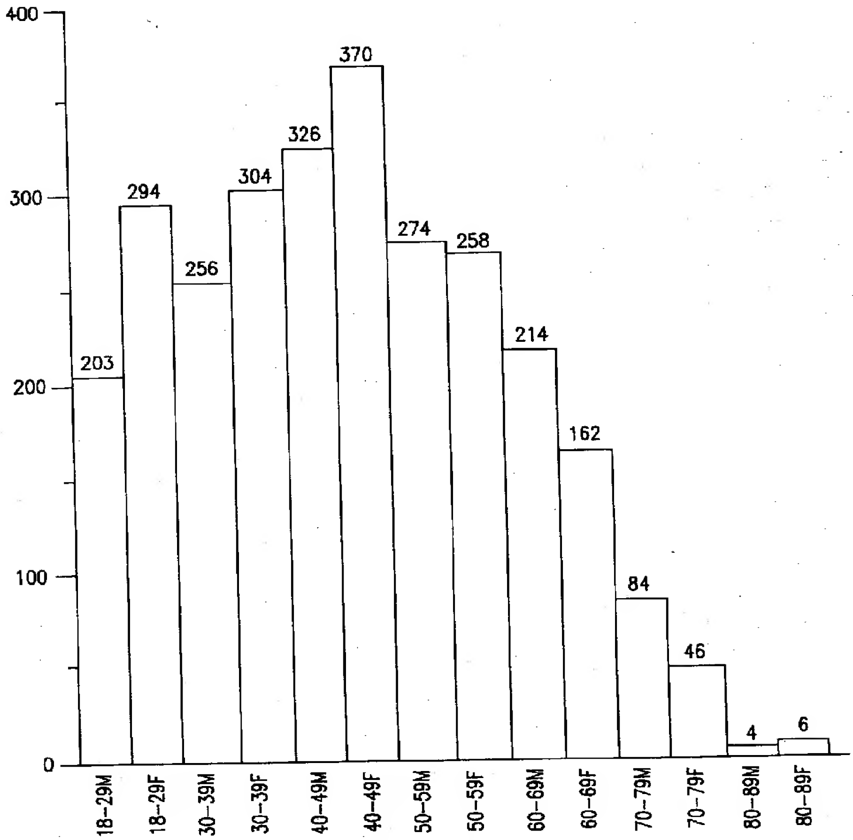


FIG. 1B

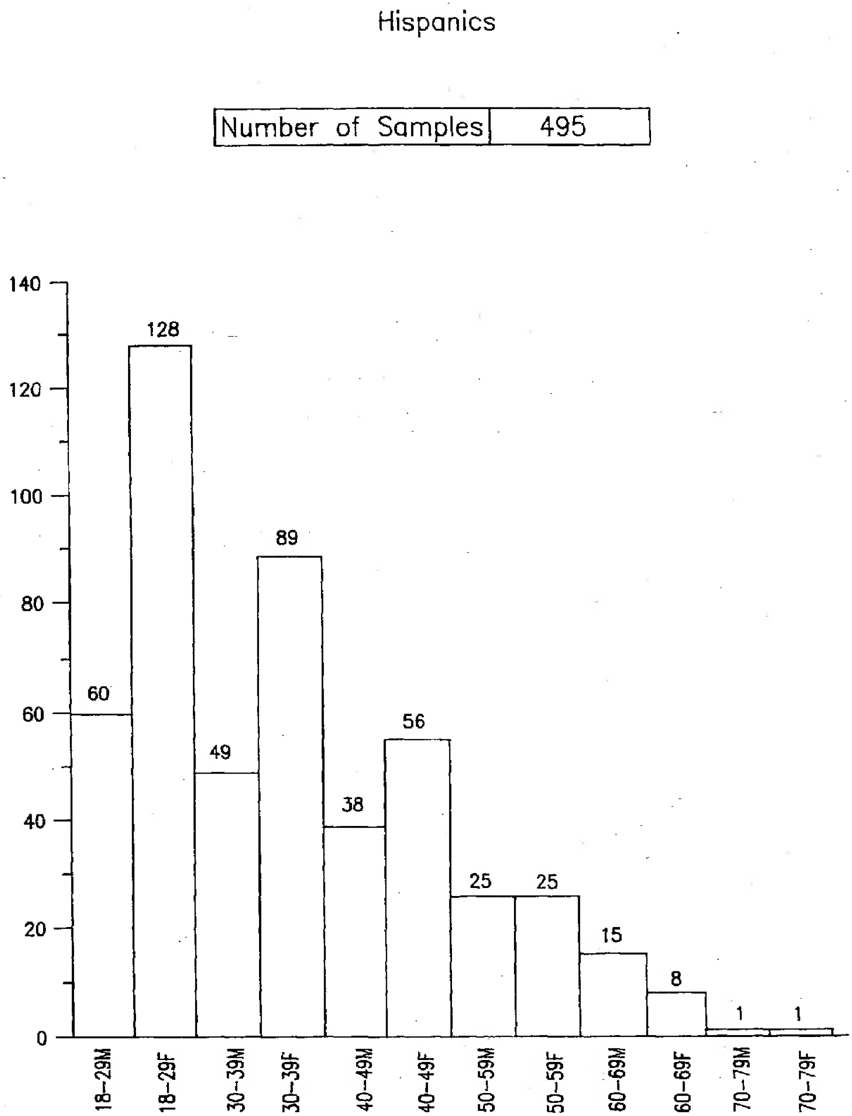
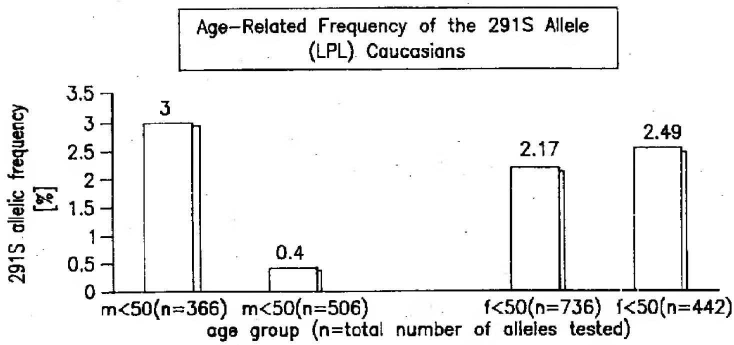
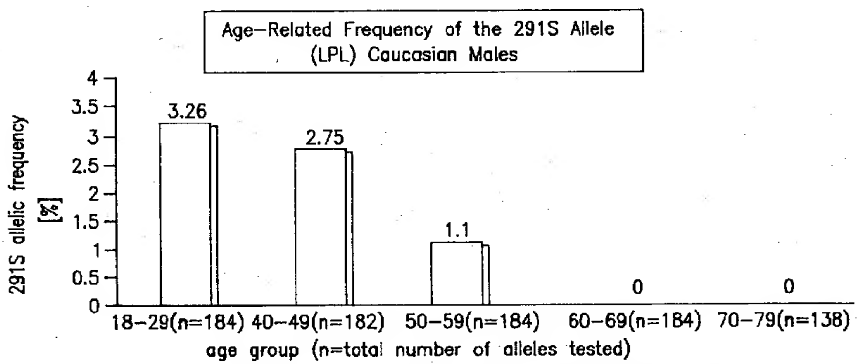

Hispanics
Number of Samples
495
140
120
100
80
60
40
20
0
128
89
60
56
49
38
25
25
15
8
1
1
18–29M
18–29F
30–39M
30–39F
40–49M
40–49F
50–59M
50–59F
60–69M
60–69F
70–79M
70–79F

FIG. IC



age- and sex-distribution of the 291S allele of the lipoprotein lipase gene. A total of 436 males and 586 females were investigated.

FIG. 2A



Age- related distribution of the 291S allele of the lipoprotein lipase gene within the male Caucasian population. A total of 436 males were tested.

FIG. 2B

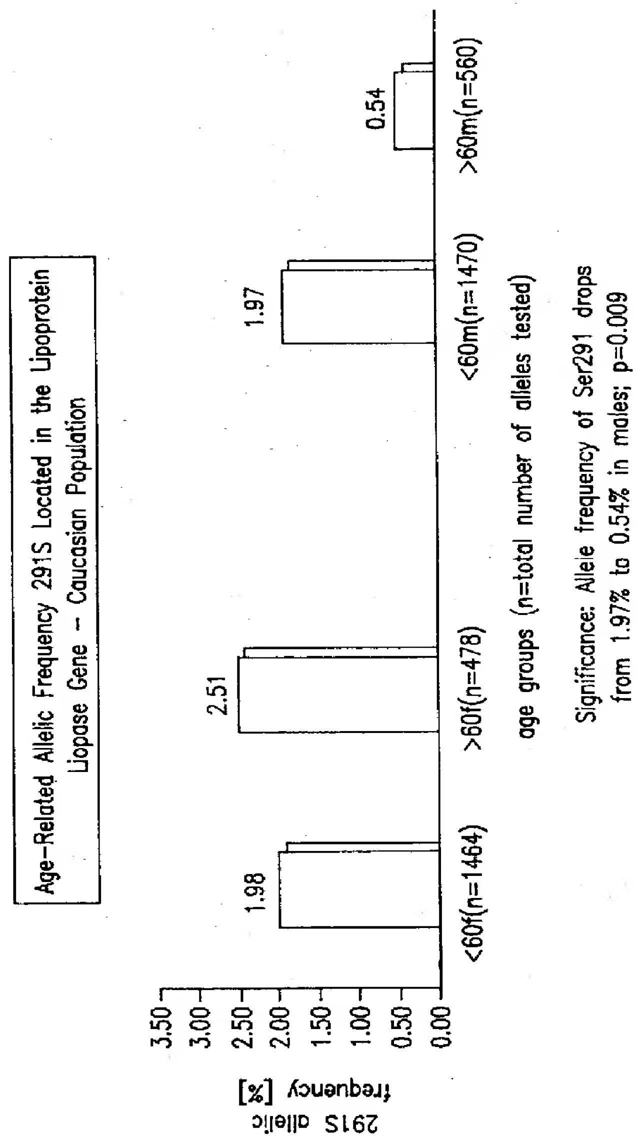

Age-Related Allelic Frequency 291S Located in the Lipoprotein Lipase Gene - Caucasian Population
291S allelic frequency [%]
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
1.98
2.51
1.97
0.54
<60f(n=1464)
>60f(n=478)
<60m(n=1470)
>60m(n=560)
age groups (n=total number of alleles tested)
Significance: Allele frequency of Ser291 drops from 1.97% to 0.54% in males; p=0.009

FIG. 2C

Questionnaire for
Population-Based
Sample Banking

Data Collection Form

Collection Information

Affix Barcode Here

Consent Form Signed Yes No
Date of Collection (MM/DD/YY)__/__/98
Time of Sample Collection(nearest hour in 24 hour clock format)________
Initials of Data Collector________Collecting Agency____________________
(DO NOT COMPLETE: (For Date Entry Only)Sample_______intact______lost ______broken

Donor information

Sex: ☐ Male ☐ Female   Date of Birth (MM/YY)__/__
In which state do you live? ______________   How long have you lived there ? _________ Years
What is your highest grade you completed in school?
☐ less than 8th grade ☐ 8th,9th,10th or 11th grade ☐ high school graduate or equivalency
☐ some college 2 yr degree ☐ college graduate 4 yr degree ☐ post graduate education or degree

To the best of your knowledge what is the Ethnic Origin of your:

Father	Mother	
☐	☐	Caucasian (please check specific geographic area below if known)
☐	☐	Northern Europe (Austria,Denmark,Finland,France,Germany,Netherlands,Norway,Sweden,Switzerland,U.K.)
☐	☐	Southern Europe (Greece,Italy,Spain)
☐	☐	Eastern Europe (Czechoslovakia,Hungary,Poland,Russia,Yugoslavia)
☐	☐	Middle Eastern (Israel,Egypt,Iran,Iraq,Jordan,Syria, other Arab States)
☐	☐	African-American
☐	☐	Hispanic (please check specific geographic area below if known)
☐	☐	Mexico
☐	☐	Central America,South American
☐	☐	Cuba,Puerto Rico, other Caribbean
☐	☐	Asian (please check specific geographic area below if known)
☐	☐	Japanese
☐	☐	Chinese
☐	☐	Korean
☐	☐	Vietnamese
☐	☐	other Asian
☐	☐	Other ______________________________
☐	☐	Don't know

Health information: Have you or has anyone in your immediate family(parents,brothers,sisters, or your children) had the following? Check all that apply

Disease:	You	Mother	Father	Sister	Brother	Child
Heart Disease Stroke or Arteriosclerosis						
Cancer (Specify type if known)						
Alzheimer's Disease or Dementia						
Chronic inflammatory or Autoimmune Disease						
Nervous System Disease like Multiple Sclerosis						
Other (please specify)						

Additional health information details you would like to provide:

____

FIG. 3

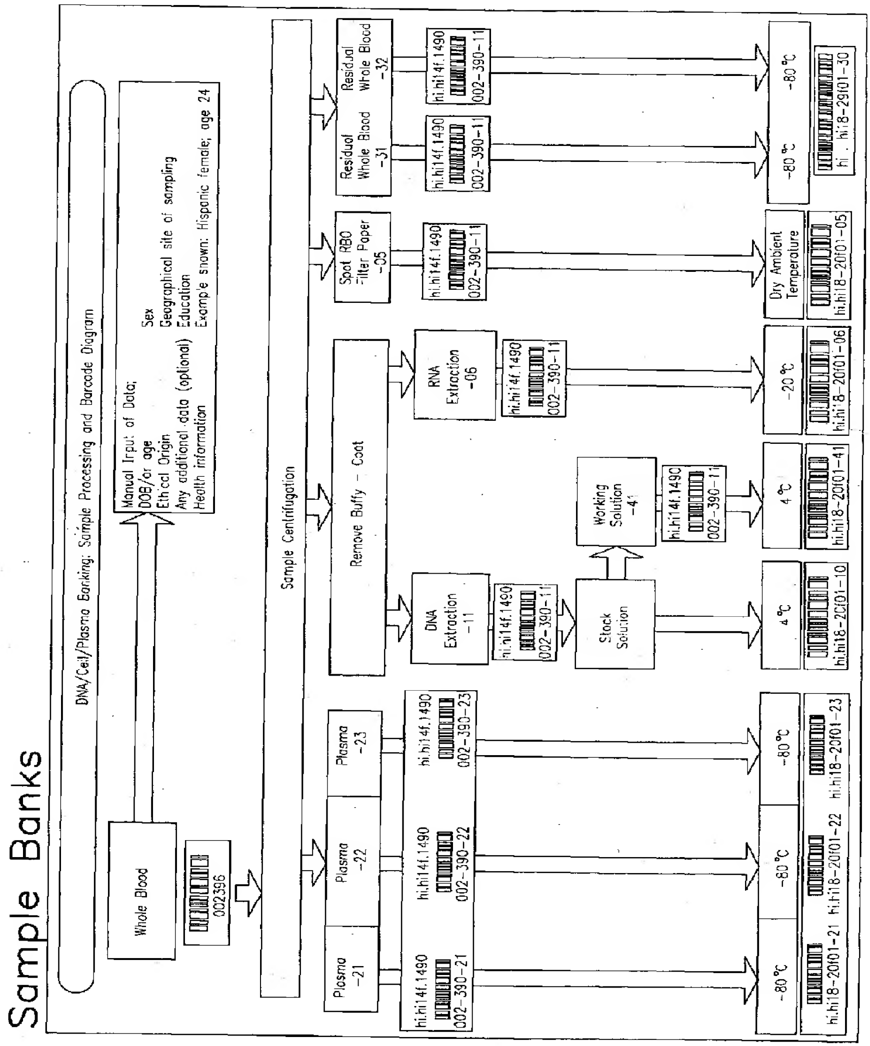

Sample Banks
DNA/Cell/Plasma Banking: Sample Processing and Barcode Diagram
Whole Blood
002396
Manual Input of Data;
DOB/or age
Ethical Origin
Any additional data (optional)
Health information
Sex
Geographical site of sampling
Education
Example shown: Hispanic female; age 24
Sample Centrifugation
Plasma -21
Plasma -22
Plasma -23
Remove Buffy - Coat
DNA Extraction -11
RNA Extraction -06
Spot RBO Filter Paper -05
Residual Whole Blood -31
Residual Whole Blood -32
Stock Solution
Working Solution -41
hi.hi14f.1490
002-390-21
002-390-22
002-390-23
002-390-11
-80 °C
-20 °C
4 °C
Dry Ambient Temperature
hi.hi18-20f01-21
hi.hi18-20f01-22
hi.hi18-20f01-23
hi.hi18-20f01-10
hi.hi18-20f01-41
hi.hi18-20f01-06
hi.hi18-20f01-05
hi.hi18-29f01-30

FIG. 4

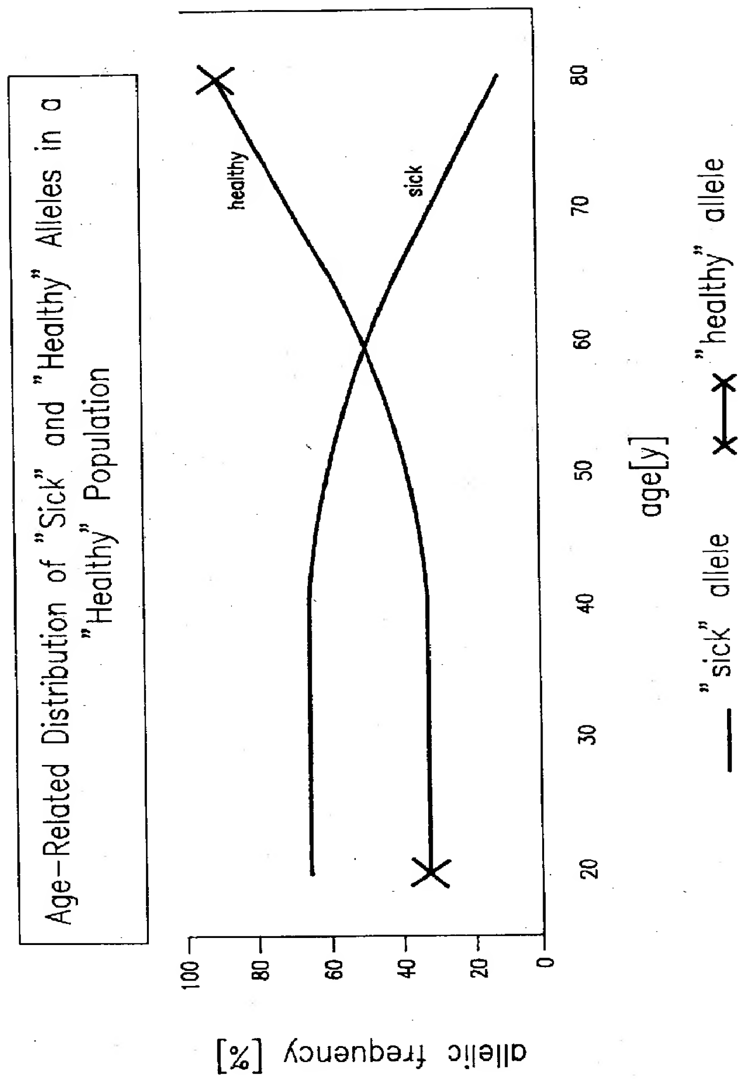

Age-Related Distribution of "Sick" and "Healthy" Alleles in a "Healthy" Population
healthy
sick
allelic frequency [%]
100
80
60
40
20
0
20
30
40
50
60
70
80
age[y]
"sick" allele
"healthy" allele

FIG. 5

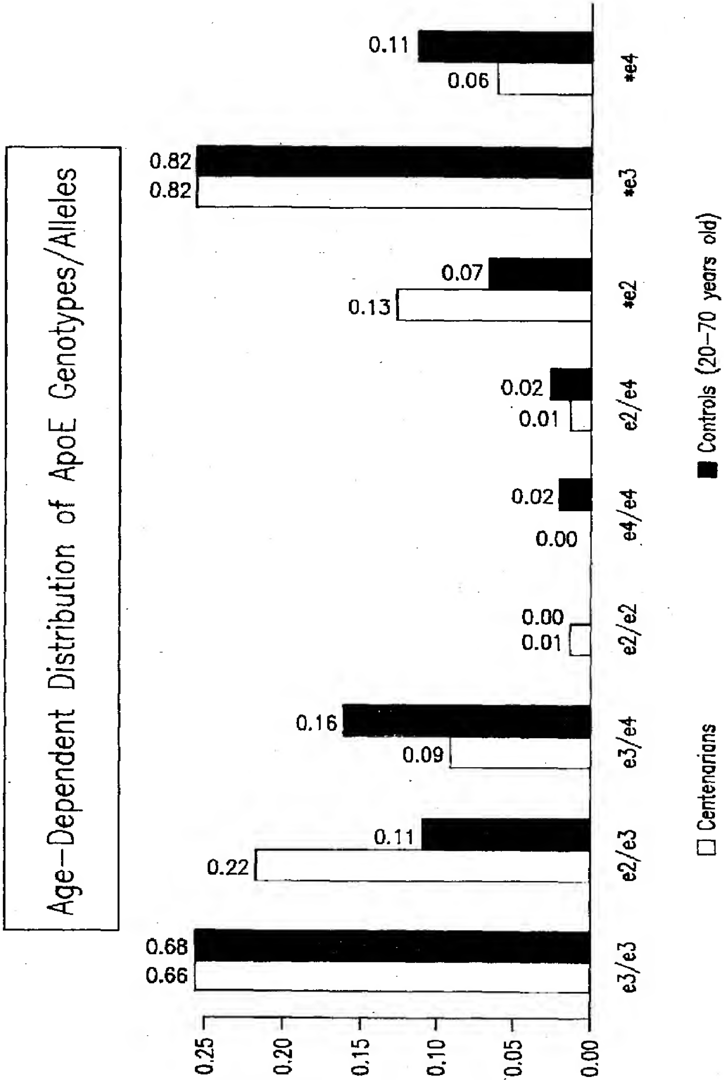

Age-Dependent Distribution of ApoE Genotypes/Alleles
0.25
0.20
0.15
0.10
0.05
0.00
e3/e3
0.68
0.66
e2/e3
0.11
0.22
e3/e4
0.16
0.09
e2/e2
0.00
0.01
e4/e4
0.02
0.00
e2/e4
0.02
0.01
*e2
0.07
0.13
*e3
0.82
0.82
*e4
0.11
0.06
Centenarians
Controls (20–70 years old)

FIG. 6

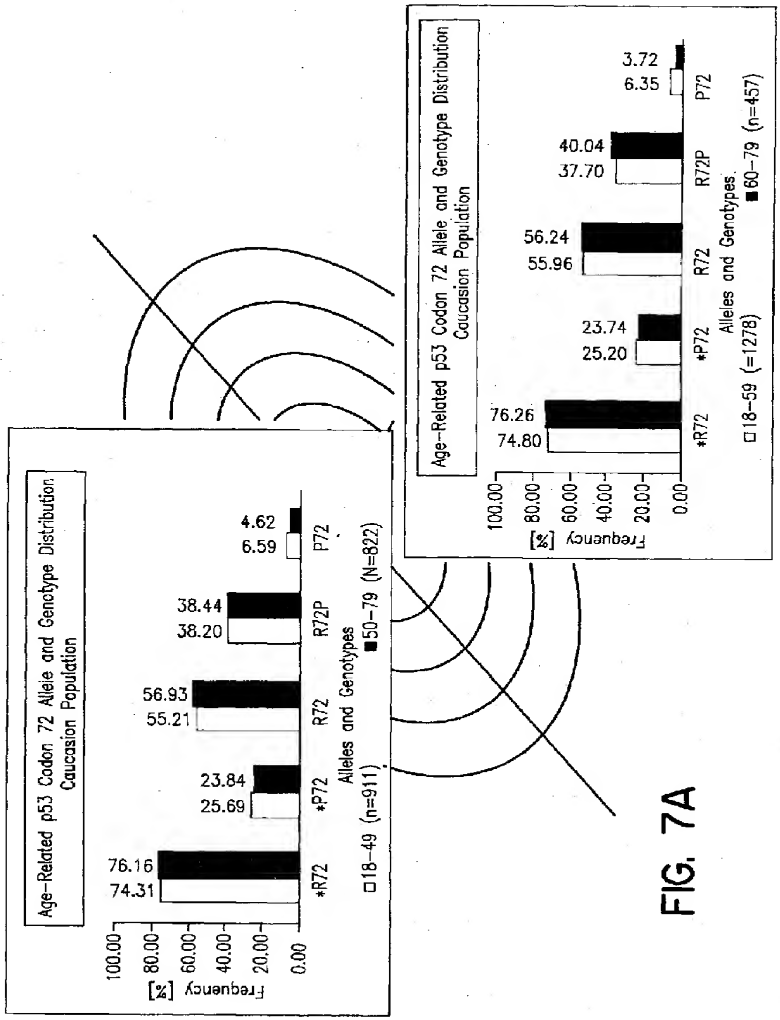
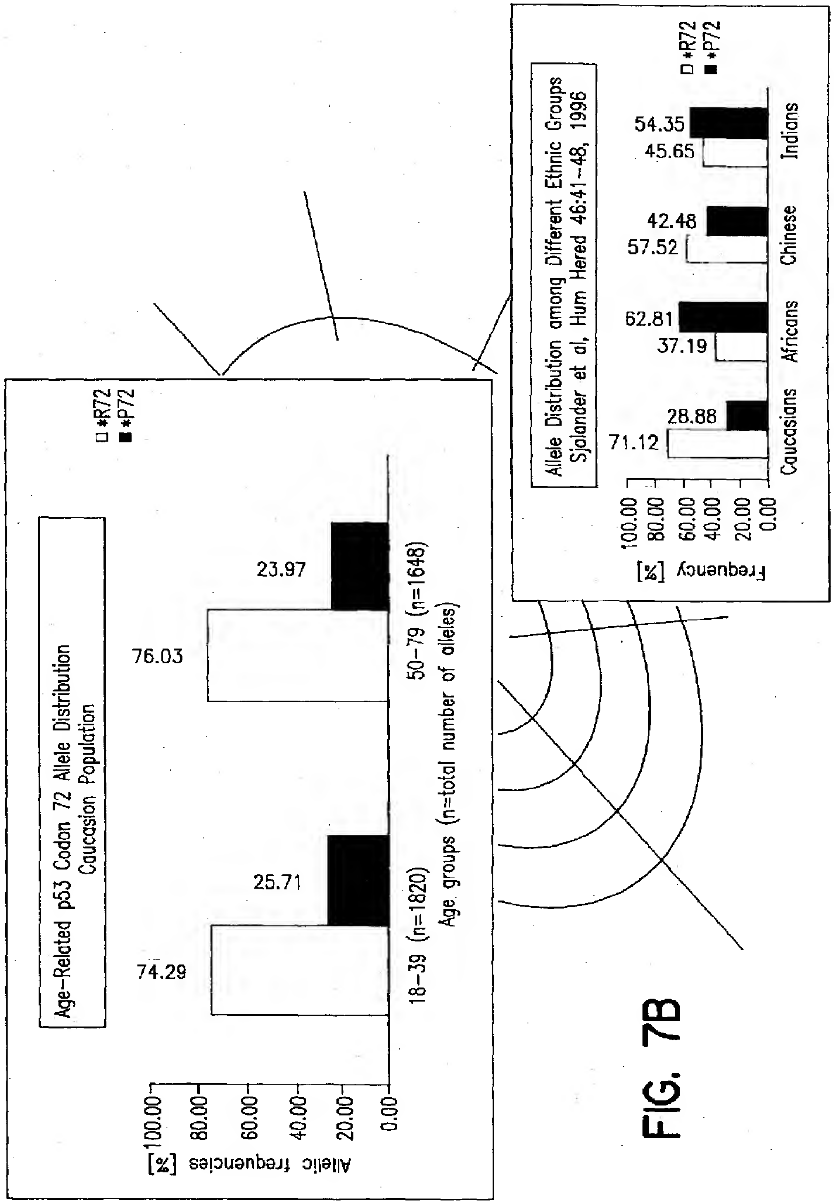

Age-Related p53 Codon 72 Allele and Genotype Distribution
Caucasion Population
Frequency [%]
100.00
80.00
60.00
40.00
20.00
0.00
76.16
74.31
23.84
25.69
56.93
55.21
38.44
38.20
4.62
6.59
*R72
*P72
R72
R72P
P72
Alleles and Genotypes
□18-49 (n=911)
■50-79 (N=822)
Age-Related p53 Codon 72 Allele and Genotype Distribution
Caucasion Population
Frequency [%]
100.00
80.00
60.00
40.00
20.00
0.00
76.26
74.80
23.74
25.20
56.24
55.96
40.04
37.70
3.72
6.35
*R72
*P72
R72
R72P
P72
Alleles and Genotypes
□18-59 (=1278)
■60-79 (n=457)

FIG. 7A



Allele Distribution among Different Ethnic Groups
Sjolander et al, Hum Hered 46:41-48, 1996
□ *R72
■ *P72
Frequency [%]
100.00
80.00
60.00
40.00
20.00
0.00
71.12
28.88
37.19
62.81
57.52
42.48
45.65
54.35
Caucasians
Africans
Chinese
Indians
Age-Related p53 Codon 72 Allele Distribution
Caucasion Population
□ *R72
■ *P72
Allelic frequencies [%]
100.00
80.00
60.00
40.00
20.00
0.00
74.29
25.71
76.03
23.97
18-39 (n=1820)
50-79 (n=1648)
Age groups (n=total number of alleles)

FIG. 7B

FIG. 7C

P53 PP vs. PR/RR Genotype Distribution
By Age cut point = 59

Age Group	N	Genotype Freq. (%) PP	PR/RR
18–59	1278	6.7	93.4
90–79	457	3.7	96.3

Sample Size : 1735
χ^2: 5.2 (1 d.f.), P = 0.02

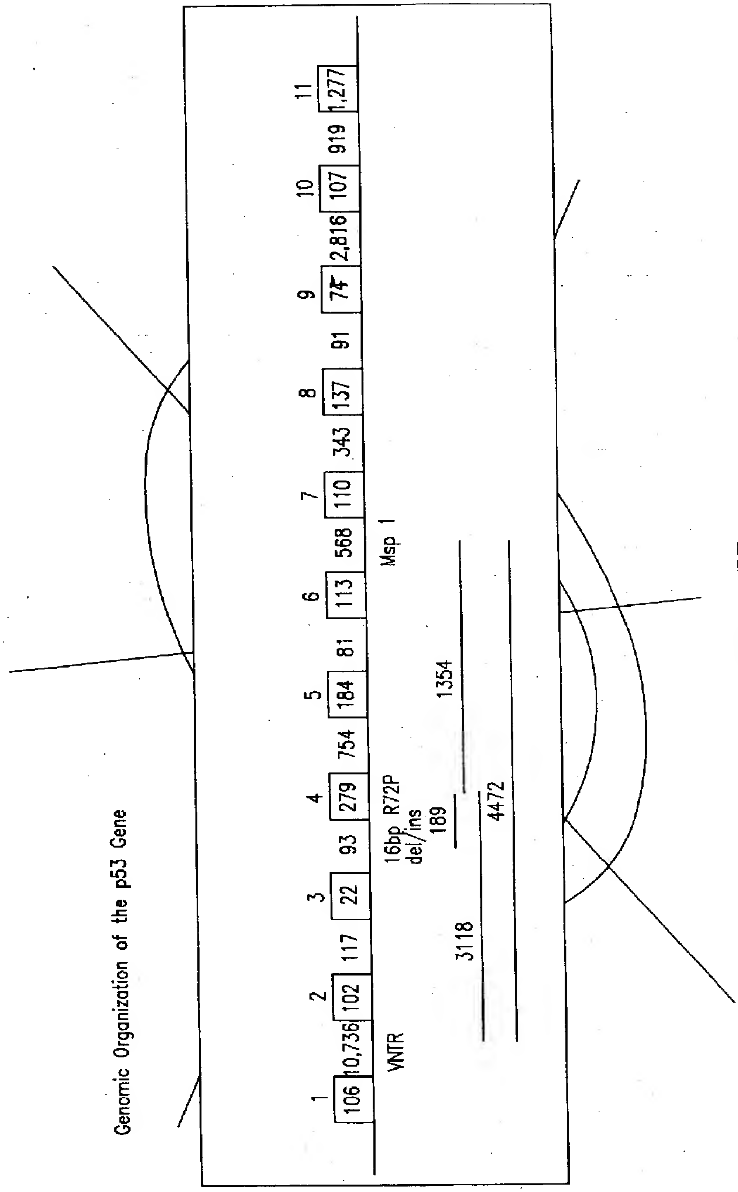

Genomic Organization of the p53 Gene
1
106
10,736
VNTR
2
102
117
3
22
93
16bp del/ins
4
279
R72P
754
5
184
81
6
113
568
Msp 1
7
110
343
8
137
91
9
74
2,816
10
107
919
11
1,277
189
1354
3118
4472

FIG. 7D

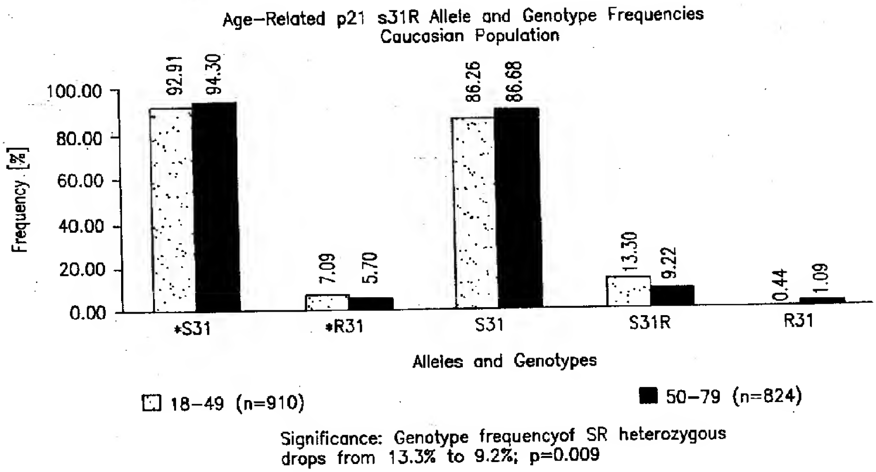

Age-Related p21 s31R Allele and Genotype Frequencies
Caucasian Population
Frequency [%]
100.00
80.00
60.00
40.00
20.00
0.00
92.91
94.30
7.09
5.70
86.26
86.68
13.30
9.22
0.44
1.09
*S31
*R31
S31
S31R
R31
Alleles and Genotypes
18-49 (n=910)
50-79 (n=824)
Significance: Genotype frequencyof SR heterozygous
drops from 13.3% to 9.2%; p=0.009

FIG. 8

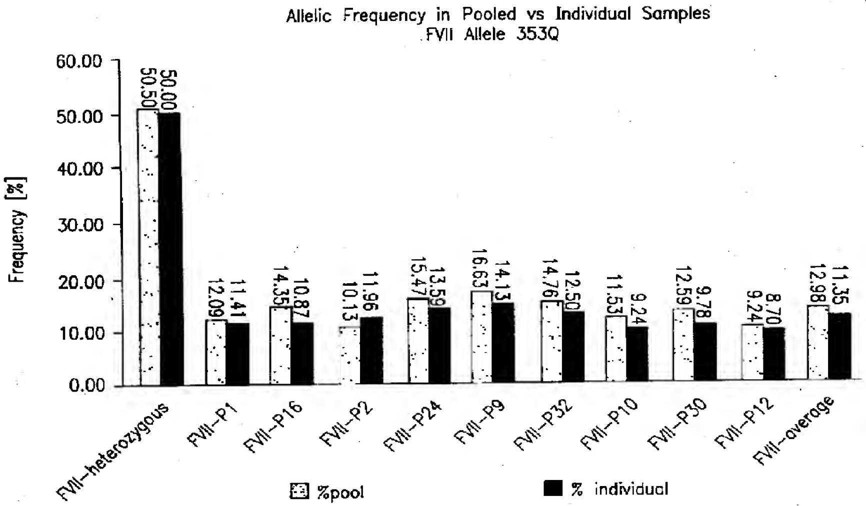
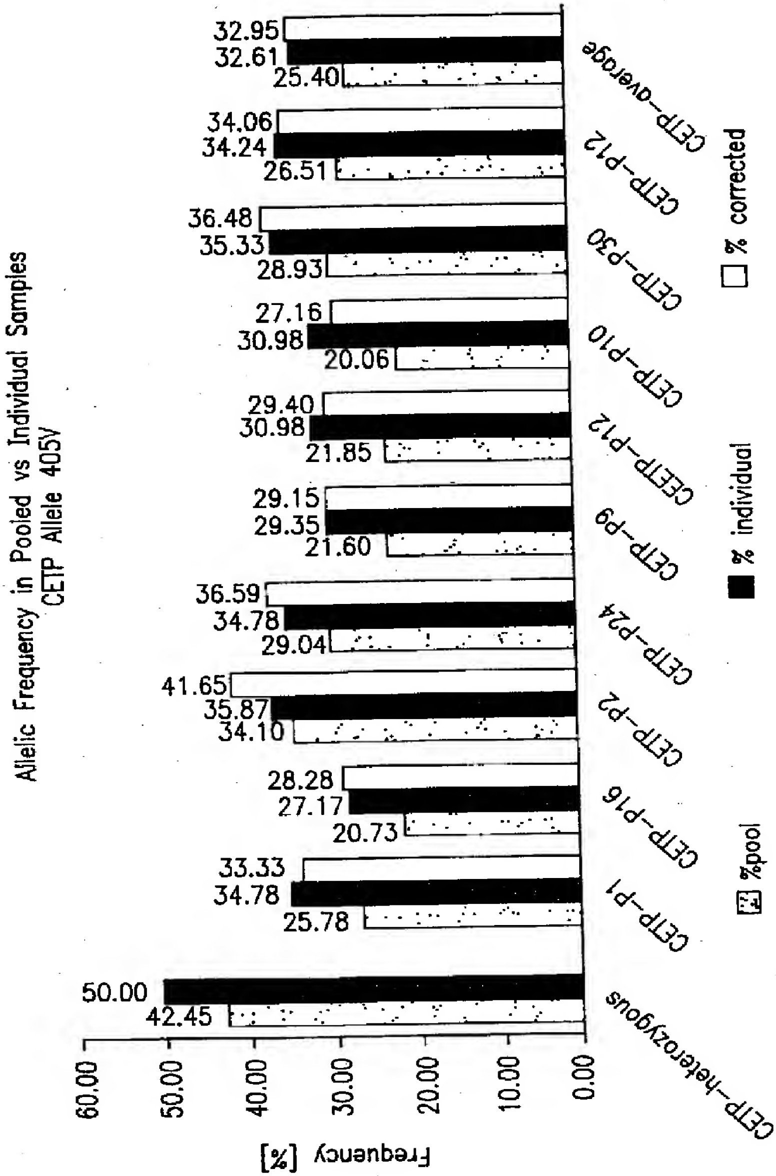
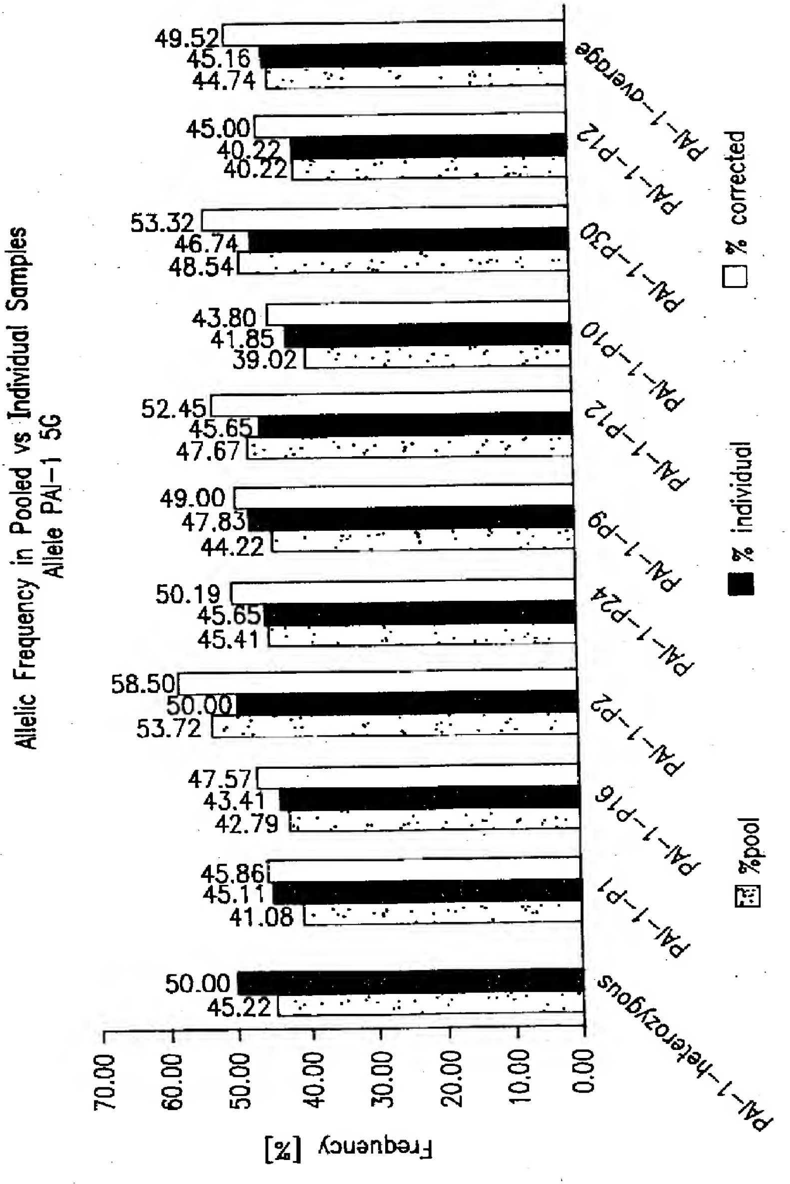

Allelic Frequency in Pooled vs Individual Samples
FVII Allele 353Q
Frequency [%]
60.00
50.00
40.00
30.00
20.00
10.00
0.00
50.50
50.00
12.09
11.41
14.35
10.87
10.13
11.96
15.47
13.59
16.63
14.13
14.76
12.50
11.53
9.24
12.59
9.78
9.24
8.70
12.98
11.35
FVII-heterozygous
FVII-P1
FVII-P16
FVII-P2
FVII-P24
FVII-P9
FVII-P32
FVII-P10
FVII-P30
FVII-P12
FVII-average
%pool
% individual

FIG. 9



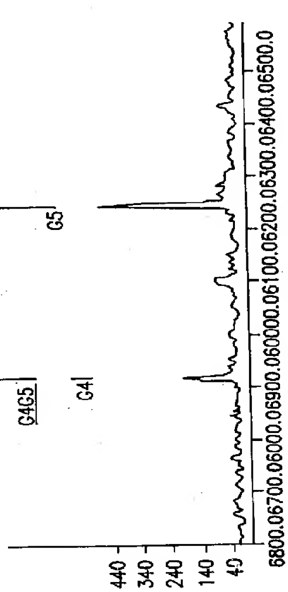
Allelic Frequency in Pooled vs Individual Samples
CETP Allele 405V
Frequency [%]
60.00
50.00
40.00
30.00
20.00
10.00
0.00
CETP-heterozygous
50.00
42.45
CETP-P1
33.33
34.78
25.78
CETP-P16
28.28
27.17
20.73
CETP-P2
41.65
35.87
34.10
CETP-P24
36.59
34.78
29.04
CETP-P9
29.15
29.35
21.60
CEETP-P12
29.40
30.98
21.85
CETP-P10
27.16
30.98
20.06
CETP-P30
36.48
35.33
28.93
CETP-P12
34.06
34.24
26.51
CETP-average
32.95
32.61
25.40
%pool
% individual
% corrected

FIG. 10



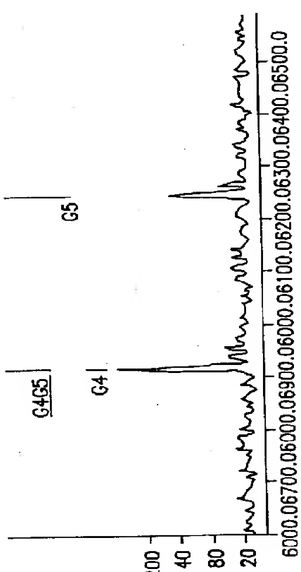
Allelic Frequency in Pooled vs Individual Samples
Allele PAI-1 5G
Frequency [%]
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
PAI-1-heterozygous
50.00
45.22
PAI-1-P1
45.86
45.11
41.08
PAI-1-P16
47.57
43.41
42.79
PAI-1-P2
58.50
50.00
53.72
PAI-1-P24
50.19
45.65
45.41
PAI-1-P9
49.00
47.83
44.22
PAI-1-P12
52.45
45.65
47.67
PAI-1-P10
43.80
41.85
39.02
PAI-1-P30
53.32
46.74
48.54
PAI-1-P12
45.00
40.22
40.22
PAI-1-average
49.52
45.16
44.74
%pool
% individual
% corrected

FIG. 11



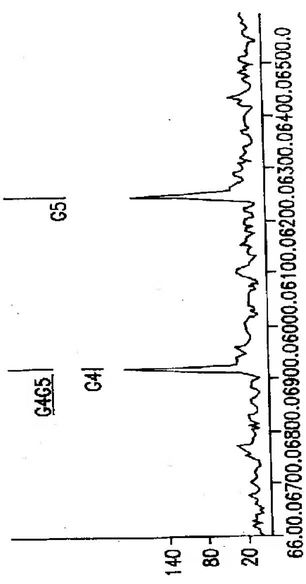
African 4G 28.2% 5G 71.2%

FIG. 12A



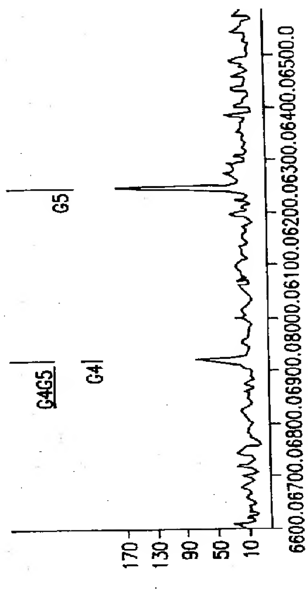
Asian 4G 65.8% 5G 34.2%

FIG. 12B



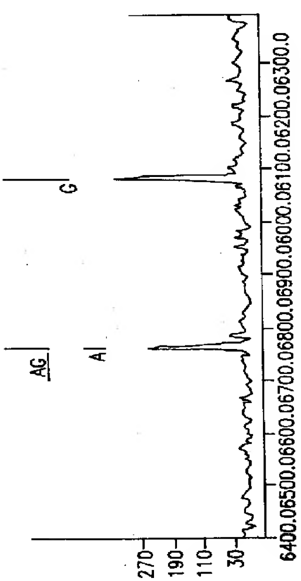
Caucasian 4G 53.5% 5G 46.5%

FIG. 12C



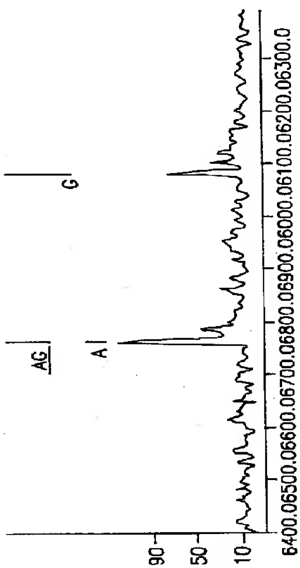
Hispanic 4G 26.7% 5G 73.3%

FIG. 12D



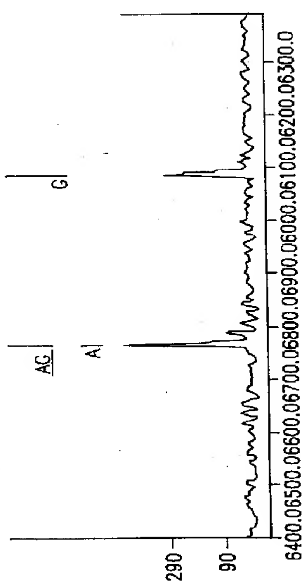
African Ile 43.6% Val 56.4%

FIG. 13A



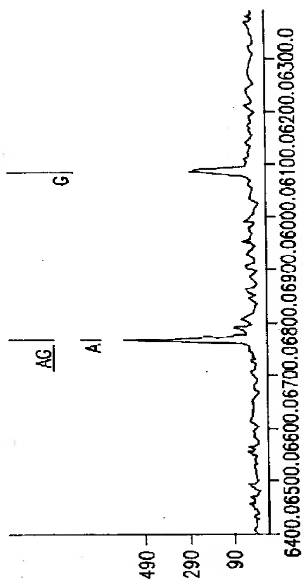
Asian Ile 60.7% Val 39.3%

FIG. 13B



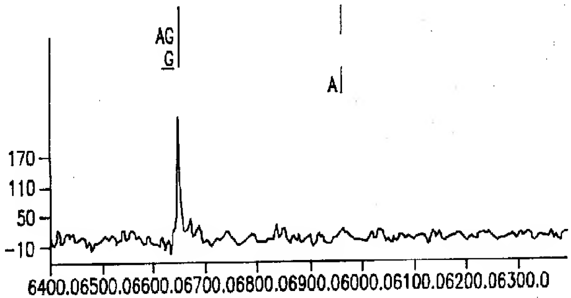
Caucasian Ile 63.0% Val 37.0%

FIG. 13C



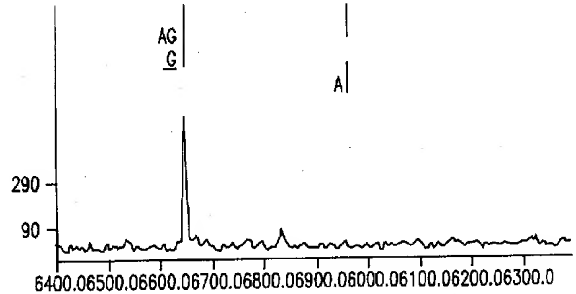
Hispanic Ile 70.1% Val 29.9%

FIG. 13D



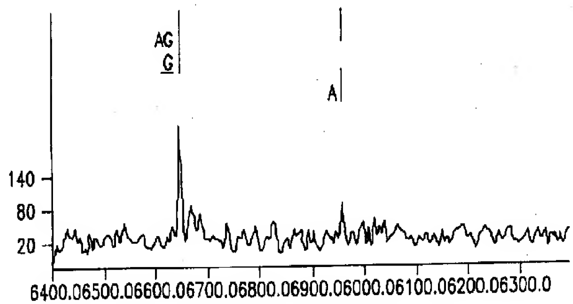
African Arg>95% Glu<5%

FIG. 14A



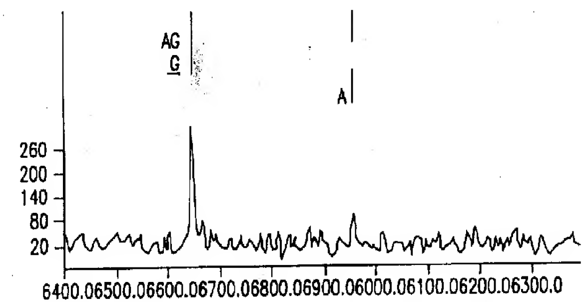
Asian Arg>95% Glu<5%

FIG. 14B



Caucasian Arg 88.5% Glu 11.5%

FIG. 14C



Hispanic Arg 89.2% Glu 10.8%

FIG. 14D

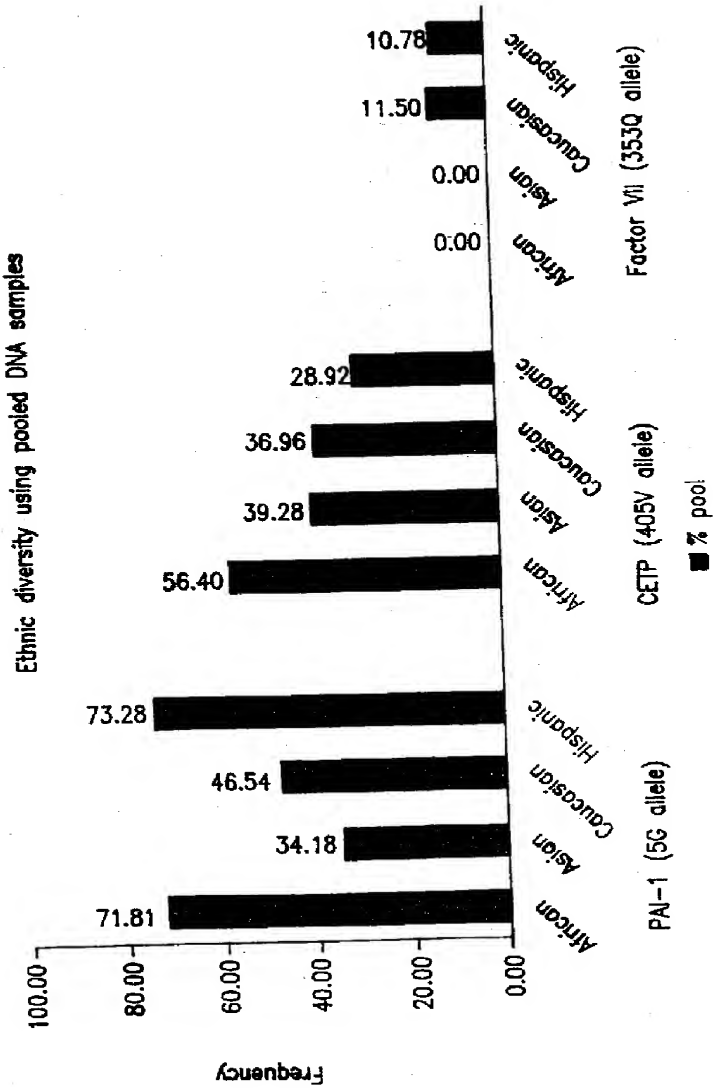

Ethnic diversity using pooled DNA samples
Frequency
100.00
80.00
60.00
40.00
20.00
0.00
71.81
34.18
46.54
73.28
56.40
39.28
36.96
28.92
0.00
0.00
11.50
10.78
African
Asian
Caucasian
Hispanic
PAI-1 (5G allele)
CETP (405V allele)
Factor VII (353Q allele)
% pool

FIG. 15

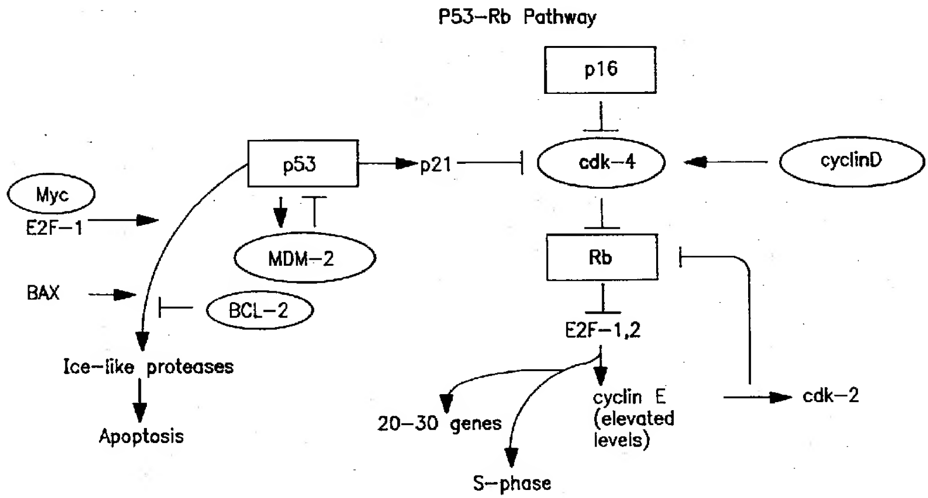

P53–Rb Pathway
p16
p53
p21
cdk–4
cyclinD
Myc
E2F–1
MDM–2
Rb
BAX
BCL–2
E2F–1,2
Ice–like proteases
Apoptosis
cyclin E
(elevated
levels)
cdk–2
20–30 genes
S–phase

FIG. 16

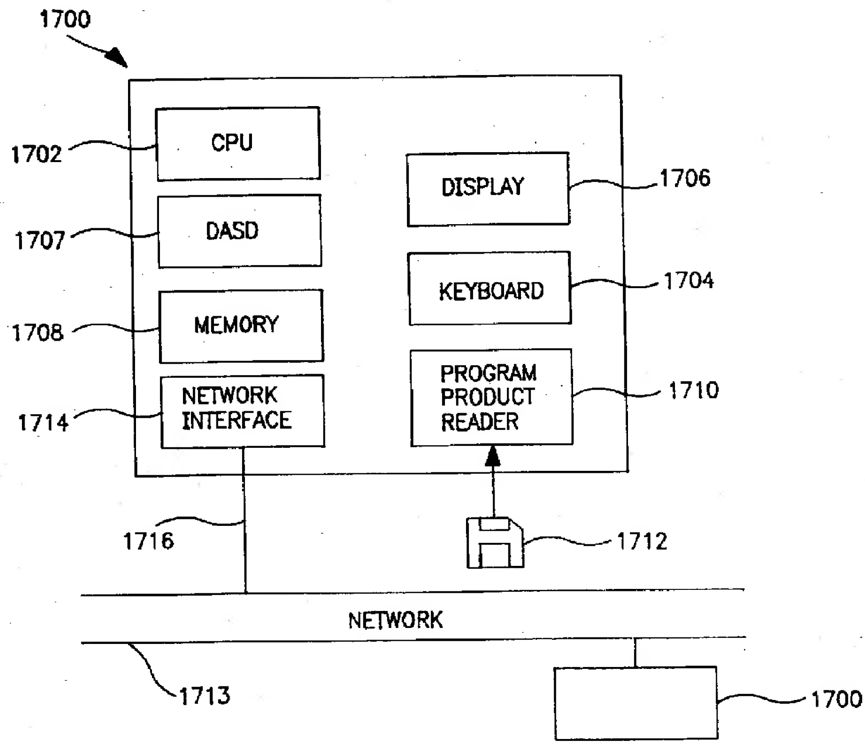

1700
1702
CPU
1707
DASD
1708
MEMORY
1714
NETWORK INTERFACE
DISPLAY
1706
KEYBOARD
1704
PROGRAM PRODUCT READER
1710
1716
1712
NETWORK
1713
1700

FIG. 17

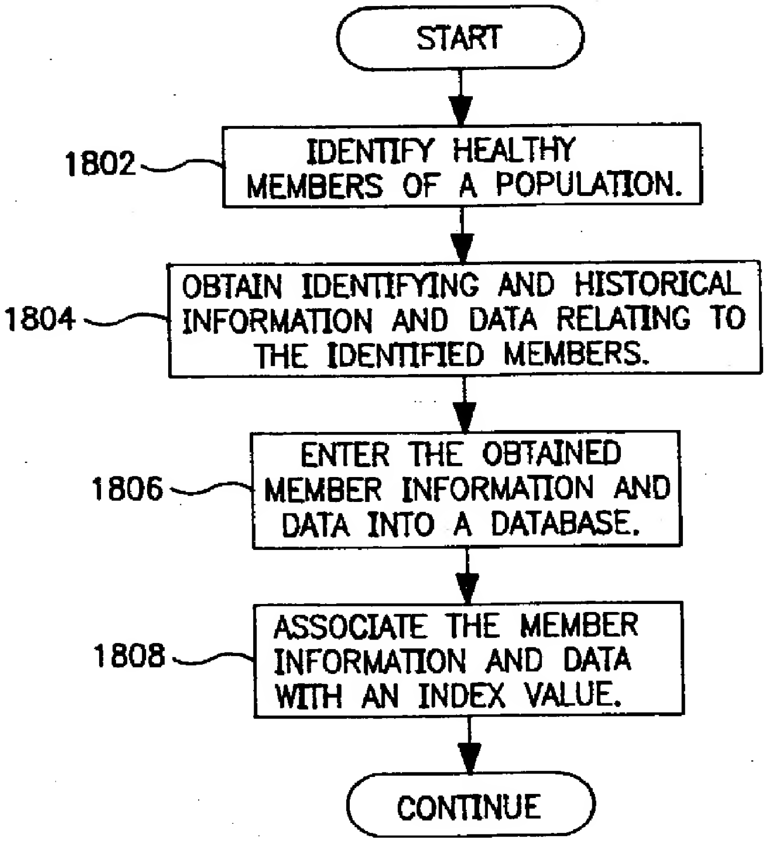

START
1802
IDENTIFY HEALTHY MEMBERS OF A POPULATION.
1804
OBTAIN IDENTIFYING AND HISTORICAL INFORMATION AND DATA RELATING TO THE IDENTIFIED MEMBERS.
1806
ENTER THE OBTAINED MEMBER INFORMATION AND DATA INTO A DATABASE.
1808
ASSOCIATE THE MEMBER INFORMATION AND DATA WITH AN INDEX VALUE.
CONTINUE

FIG. 18

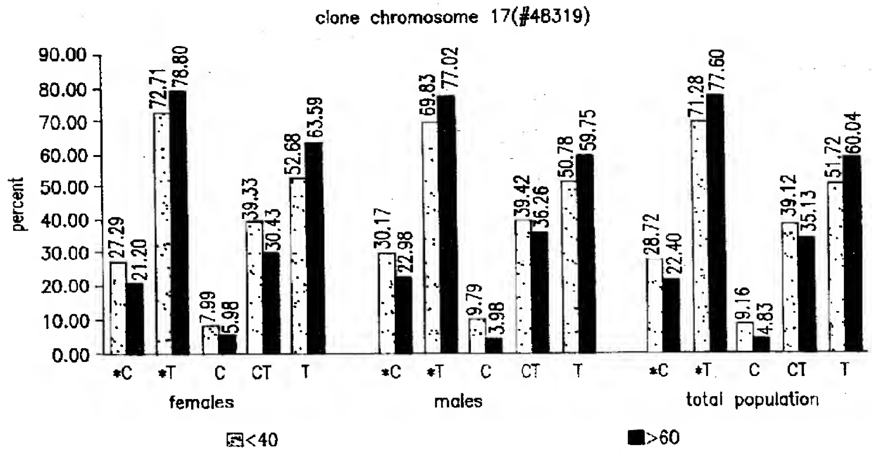

clone chromosome 17(#48319)
percent
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
27.29
21.20
72.71
78.80
7.99
5.98
39.33
30.43
52.68
63.59
30.17
22.98
69.83
77.02
9.79
3.98
39.42
36.26
50.78
59.75
28.72
22.40
71.28
77.60
9.16
4.83
39.12
35.13
51.72
60.04
*C
*T
C
CT
T
females
males
total population
<40
>60

FIG. 19

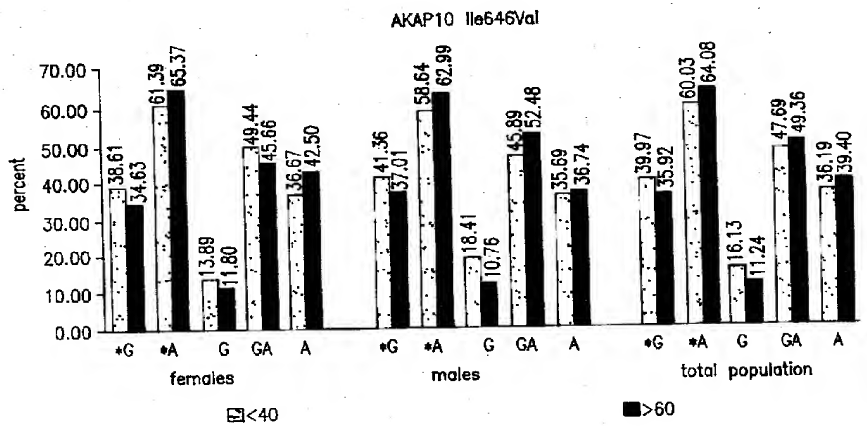

AKAP10 Ile646Val
percent
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
38.61
34.63
61.39
65.37
13.89
11.80
49.44
45.66
36.67
42.50
41.36
37.01
58.64
62.99
18.41
10.76
45.89
52.48
35.69
36.74
39.97
35.92
60.03
64.08
16.13
11.24
47.69
49.36
36.19
39.40
*G
*A
G
GA
A
females
males
total population
<40
>60

FIG. 20

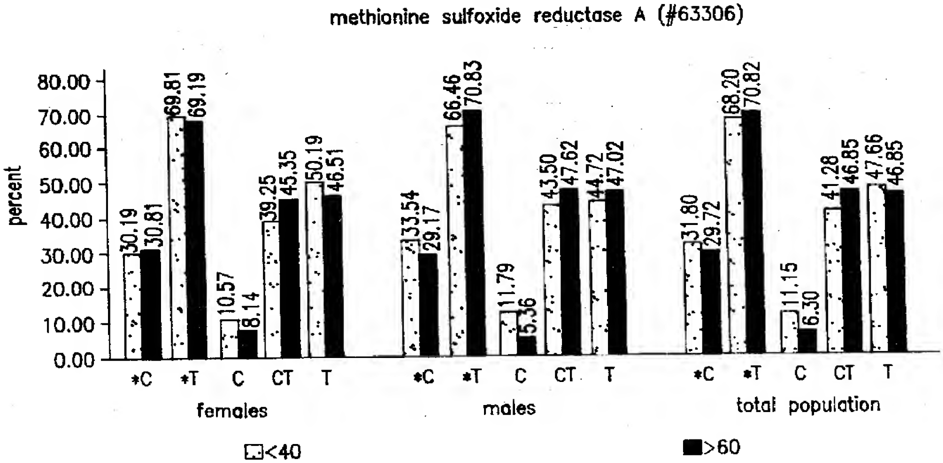

methionine sulfoxide reductase A (#63306)
percent
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
30.19
30.81
69.81
69.19
10.57
8.14
39.25
45.35
50.19
46.51
33.54
29.17
66.46
70.83
11.79
5.36
43.50
47.62
44.72
47.02
31.80
29.72
68.20
70.82
11.15
6.30
41.28
46.85
47.66
46.85
*C
*T
C
CT
T
females
males
total population
<40
>60

FIG. 21

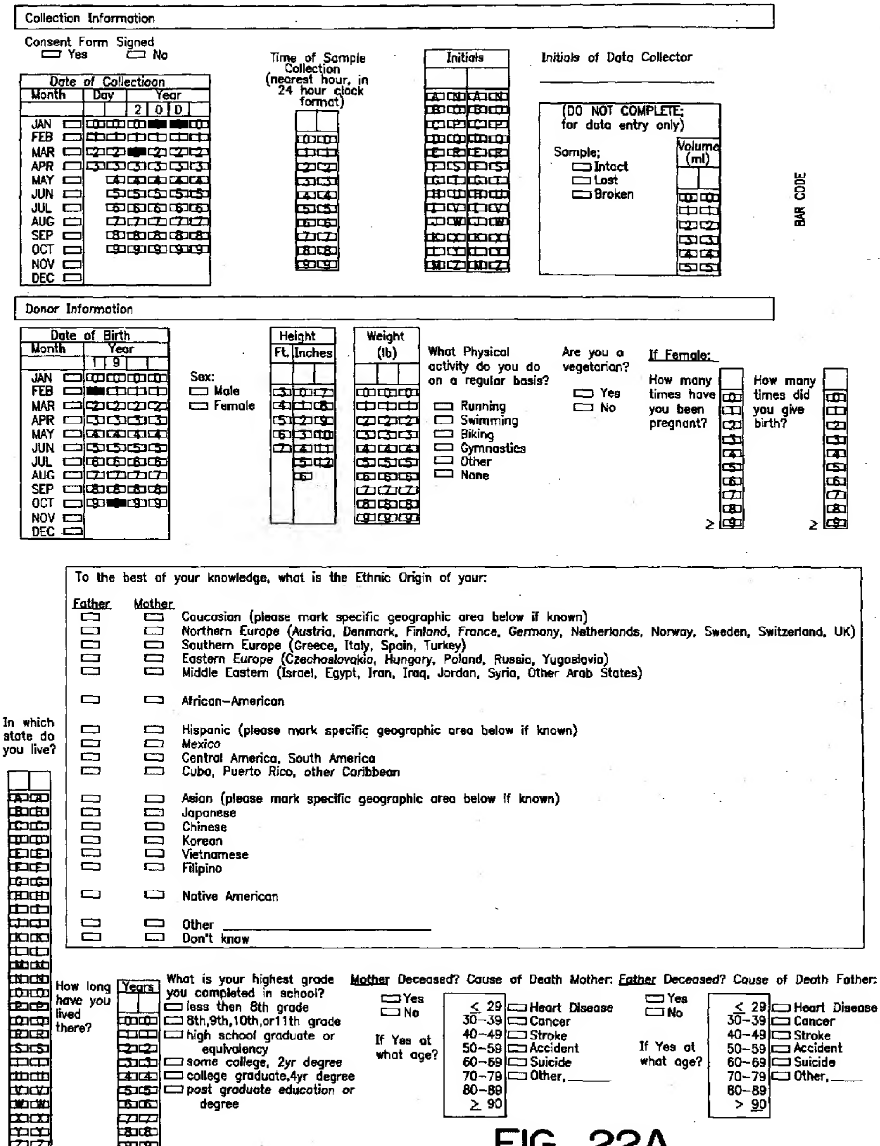

Collection Information
Consent Form Signed
Yes No
Date of Collection
Month Day Year
2 0 D
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
Time of Sample Collection (nearest hour, in 24 hour clock format)
Initials
Initials of Data Collector
(DO NOT COMPLETE; for data entry only)
Sample;
Intact
Lost
Broken
Volume (ml)
BAR CODE
Donor Information
Date of Birth
Month Year
1 9
Sex:
Male
Female
Height
Ft. Inches
Weight (lb)
What Physical activity do you do on a regular basis?
Running
Swimming
Biking
Gymnastics
Other
None
Are you a vegetarian?
Yes
No
If Female:
How many times have you been pregnant?
How many times did you give birth?
To the best of your knowledge, what is the Ethnic Origin of your:
Father Mother
Caucasian (please mark specific geographic area below if known)
Northern Europe (Austria, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, UK)
Southern Europe (Greece, Italy, Spain, Turkey)
Eastern Europe (Czechoslovakia, Hungary, Poland, Russia, Yugoslavia)
Middle Eastern (Israel, Egypt, Iran, Iraq, Jordan, Syria, Other Arab States)
African-American
Hispanic (please mark specific geographic area below if known)
Mexico
Central America, South America
Cuba, Puerto Rico, other Caribbean
Asian (please mark specific geographic area below if known)
Japanese
Chinese
Korean
Vietnamese
Filipino
Native American
Other
Don't know
In which state do you live?
How long have you lived there?
Years
What is your highest grade you completed in school?
less then 8th grade
8th,9th,10th,or11th grade
high school graduate or equivalency
some college, 2yr degree
college graduate,4yr degree
post graduate education or degree
Mother Deceased?
Yes
No
If Yes at what age?
Cause of Death Mother:
≤ 29
30-39
40-49
50-59
60-69
70-79
80-89
≥ 90
Heart Disease
Cancer
Stroke
Accident
Suicide
Other,
Father Deceased?
Yes
No
If Yes at what age?
Cause of Death Father:
≤ 29
30-39
40-49
50-59
60-69
70-79
80-89
> 90
Heart Disease
Cancer
Stroke
Accident
Suicide
Other,

FIG. 22A

Have you ever smoked? ▭ Yes ▭ No

If yes, for how long? Years

0 0
1 1
2 2
3 3
4 4
5 5
6 6
7 7
8 8
9 9

Have you been hospitalized in the past 5 years for more then 6 days at a time?
▭ Yes ▭ No

If yes, how many times?
1 2 3 4 5 6 7 8 9

For each hospitalization (if not the same) how long did you stay and for what reason?

1) Weeks: 1 2 3 4 5 6
▭ Acute disorder, including infection and thrombosis
▭ Chronic Disorder
▭ Accident
▭ Other:________

2) Weeks: 1 2 3 4 5 6
▭ Acute disorder, including infection and thrombosis
▭ Chronic Disorder
▭ Accident
▭ Other:________

3) Weeks: 1 2 3 4 5 6
▭ Acute disorder, including infection and thrombosis
▭ Chronic Disorder
▭ Accident
▭ Other:________

Have you or has anyone in your immediate family (parents,brothers,sisters,or your children) had the following?
Mark all that apply!

Disease	You	Mother	Father	Sister	Brother	Child
Heart Disease, including arteriosclerosis	▭	▭	▭	▭	▭	▭
Stroke	▭	▭	▭	▭	▭	▭
Hypertension	▭	▭	▭	▭	▭	▭
Blood clots	▭	▭	▭	▭	▭	▭
Diabetes, insulin dependent	▭	▭	▭	▭	▭	▭
Diabetes, not insulin-dependent (diet controlled)	▭	▭	▭	▭	▭	▭
Cancer:	▭	▭	▭	▭	▭	▭
Lung&Bronchus	▭	▭	▭	▭	▭	▭
Breasts	▭	▭	▭	▭	▭	▭
Prostate	▭	▭	▭	▭	▭	▭
Colon&Rectum	▭	▭	▭	▭	▭	▭
Skin	▭	▭	▭	▭	▭	▭
Lymphoma&Leukemia	▭	▭	▭	▭	▭	▭
Other, please specify below:	▭	▭	▭	▭	▭	▭
Alzheimer's Disease	▭	▭	▭	▭	▭	▭
Epilepsy	▭	▭	▭	▭	▭	▭
Schizophrenia	▭	▭	▭	▭	▭	▭
Bipolar disorder (manic depression)	▭	▭	▭	▭	▭	▭
Major depression	▭	▭	▭	▭	▭	▭
Chronic Inflammatory or Autoimmune Disease including Multiple Sclerosis and Rheumatoid Arthritis	▭	▭	▭	▭	▭	▭
Emphysema	▭	▭	▭	▭	▭	▭
Asthma	▭	▭	▭	▭	▭	▭
Other, please specify below:						

Do you take prescription drugs on a regular basis? ▭ Yes ▭ No

If yes, please specify below:

Have you ever donated blood before? ▭ Yes ▭ No

If yes, how many times: Number of Times

0 0 0
1 1 1
2 2 2
3 3 3
4 4 4
5 5 5
6 6 6
7 7 7
8 8 8
9 9 9

Additional health information details you would like to provide:

Do you drink any kind of alcoholic beverage?
▭ Never ▭ Hardly ever
▭ Less than 3 times per week ▭ 3 or more times per week
▭ Daily

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■	6
2	7
3	8
4	9
5	0

FIG. 22B

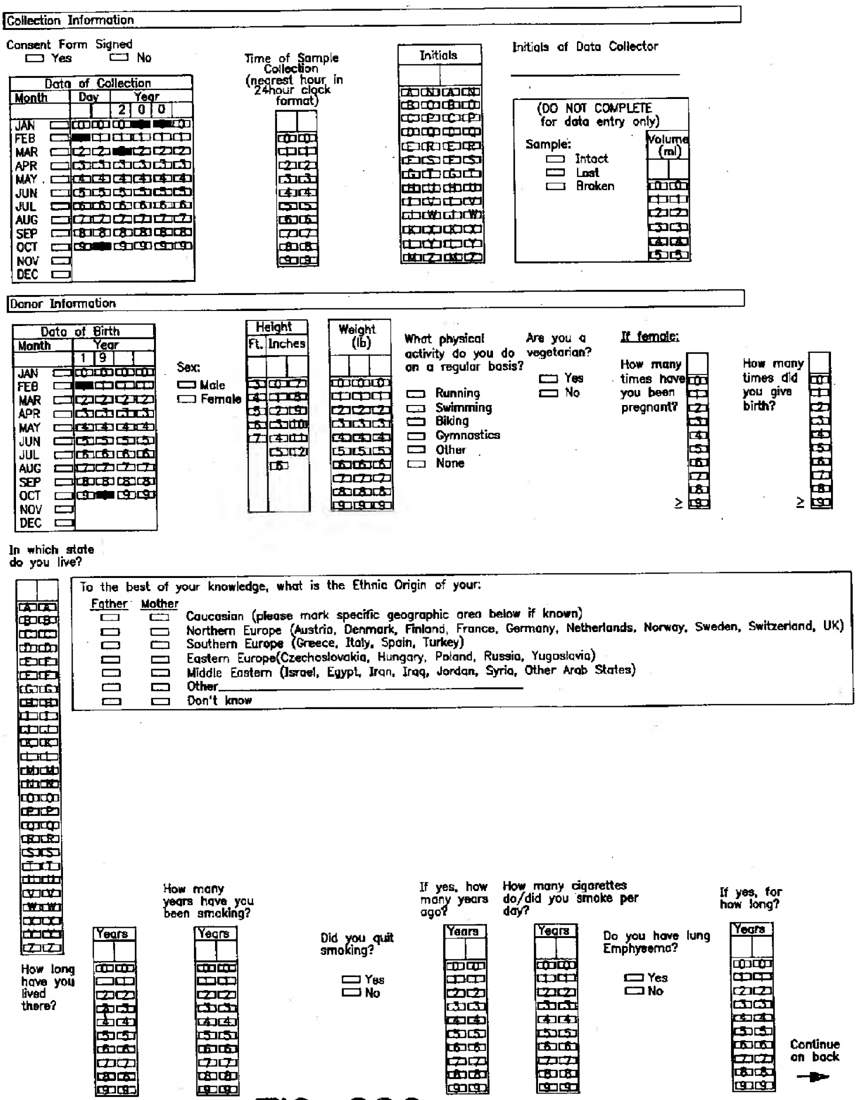
Collection Information
Consent Form Signed
Yes
No
Date of Collection
Month
Day
Year
2 0 0
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
Time of Sample Collection (nearest hour in 24hour clock format)
Initials
Initials of Data Collector
(DO NOT COMPLETE for data entry only)
Sample:
Intact
Lost
Broken
Volume (ml)
Donor Information
Date of Birth
Month
Year
1 9
Sex:
Male
Female
Height
Ft.
Inches
Weight (lb)
What physical activity do you do on a regular basis?
Running
Swimming
Biking
Gymnastics
Other
None
Are you a vegetarian?
Yes
No
If female:
How many times have you been pregnant?
How many times did you give birth?
In which state do you live?
To the best of your knowledge, what is the Ethnic Origin of your:
Father
Mother
Caucasian (please mark specific geographic area below if known)
Northern Europe (Austria, Denmark, Finland, France, Germany, Netherlands, Norway, Sweden, Switzerland, UK)
Southern Europe (Greece, Italy, Spain, Turkey)
Eastern Europe(Czechoslovakia, Hungary, Poland, Russia, Yugoslavia)
Middle Eastern (Israel, Egypt, Iran, Iraq, Jordan, Syria, Other Arab States)
Other
Don't know
How long have you lived there?
Years
How many years have you been smoking?
Did you quit smoking?
Yes
No
If yes, how many years ago?
How many cigarettes do/did you smoke per day?
Do you have lung Emphysema?
If yes, for how long?
Continue on back

FIG. 22C

What is your highest grade you completed in school?
- less then 8th grade
- 8th,9th,10th,or11th grade
- high school graduate or equivalency
- some college, 2yr degree
- college graduate,4yr degree
- post graduate education or degree

Mother Deceased? Yes / No. If Yes at what age? ≤ 29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–89, ≥ 90

Cause of Death Mother: Heart Disease, Cancer, Stroke, Accident, Suicide, Other, ________

Father Deceased? Yes / No. If Yes at what age? ≤ 29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–89, > 90

Cause of Death Father: Heart Disease, Cancer, Stroke, Accident, Suicide, Other, ________

Health Information

Have you or has anyone in your immediate family (parents,brothers,sisters,or your children) had the following? Mark all that apply!

Disease	You	Mother	Father	Sister	Brother	Child
Heart Disease	☐	☐	☐	☐	☐	☐
Stroke	☐	☐	☐	☐	☐	☐
Hypertension	☐	☐	☐	☐	☐	☐
Blood clots	☐	☐	☐	☐	☐	☐
Diabetes, insulin dependent	☐	☐	☐	☐	☐	☐
Diabetes, not insulin-dependent	☐	☐	☐	☐	☐	☐
Cancer:	☐	☐	☐	☐	☐	☐
Lung&Bronchus	☐	☐	☐	☐	☐	☐
Breasts	☐	☐	☐	☐	☐	☐
Prostate	☐	☐	☐	☐	☐	☐
Colon&Rectum	☐	☐	☐	☐	☐	☐
Skin	☐	☐	☐	☐	☐	☐
Lymphoma&Leukemia	☐	☐	☐	☐	☐	☐
Other, please specify below:	☐	☐	☐	☐	☐	☐
Alzheimer's Disease	☐	☐	☐	☐	☐	☐
Epilepsy	☐	☐	☐	☐	☐	☐
Schizophrenia	☐	☐	☐	☐	☐	☐
Bipolar disorder (manic depression)	☐	☐	☐	☐	☐	☐
Major depression	☐	☐	☐	☐	☐	☐
Chronic Inflammatory or Autoimmune Disease including Multiple Sclerosis and Rheumatoid Arthritis	☐	☐	☐	☐	☐	☐
Emphysema	☐	☐	☐	☐	☐	☐
Asthma	☐	☐	☐	☐	☐	☐
Other, please specify below:						

Do you take prescription drugs on a regular basis? Yes / No

If yes, please specify below:

Have you ever donated blood before? Yes / No

If yes, how many times? Number of Times: 0–9

Have you been hospitalized in the past 5 years for more then 6 days at a time? Yes / No

If yes, how many times? 1 2 3 4 5 6 7 8 9

For each hospitalization (if not the same) how long did you stay and for what reason?

1) Weeks: 1 2 3 4 5 6
- Acute disorder, including infection and thrombosis
- Chronic Disorder
- Accident
- Other: ________

2) Weeks: 1 2 3 4 5 6
- Acute disorder, including infection and thrombosis
- Chronic Disorder
- Accident
- Other: ________

3) Weeks: 1 2 3 4 5 6
- Acute disorder, including infection and thrombosis
- Chronic Disorder
- Accident
- Other: ________

Do you drink any kind of alcoholic beverage?
- Never
- Hardly ever
- Less than 3 times per week
- 3 or more times per week
- Daily

Additional health information details you would like to provide:

____

____

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1 6
2 7
3 8
4 9
5 0

FIG. 22D

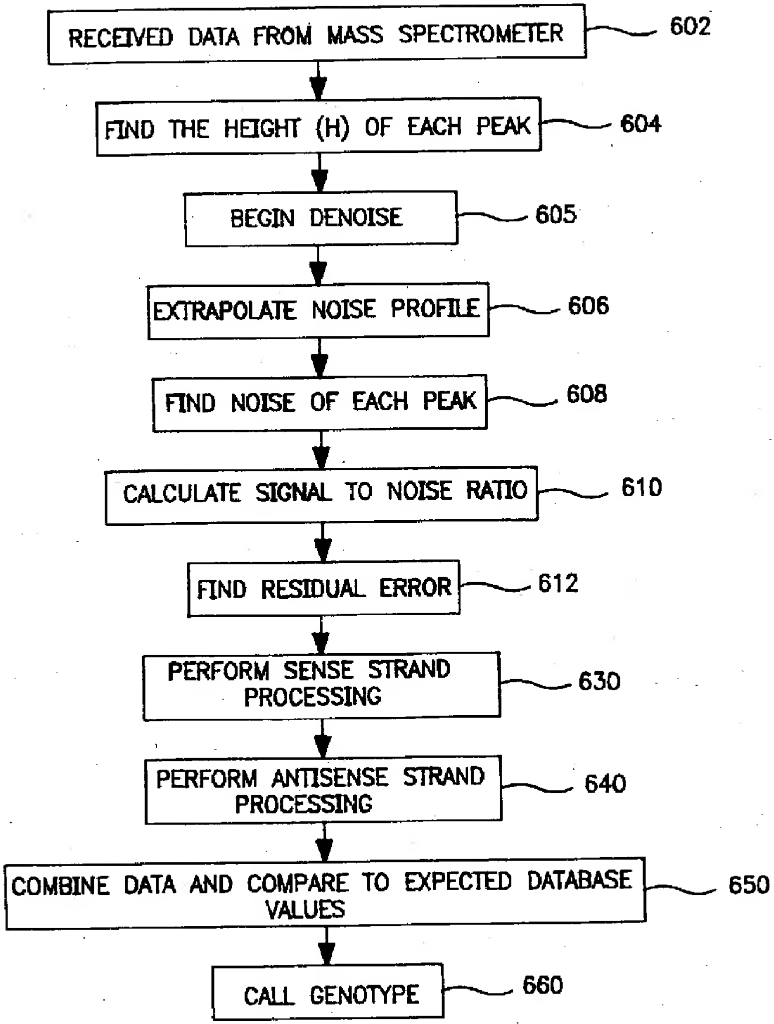

RECEIVED DATA FROM MASS SPECTROMETER
602
FIND THE HEIGHT (H) OF EACH PEAK
604
BEGIN DENOISE
605
EXTRAPOLATE NOISE PROFILE
606
FIND NOISE OF EACH PEAK
608
CALCULATE SIGNAL TO NOISE RATIO
610
FIND RESIDUAL ERROR
612
PERFORM SENSE STRAND PROCESSING
630
PERFORM ANTISENSE STRAND PROCESSING
640
COMBINE DATA AND COMPARE TO EXPECTED DATABASE VALUES
650
CALL GENOTYPE
660

FIG. 23

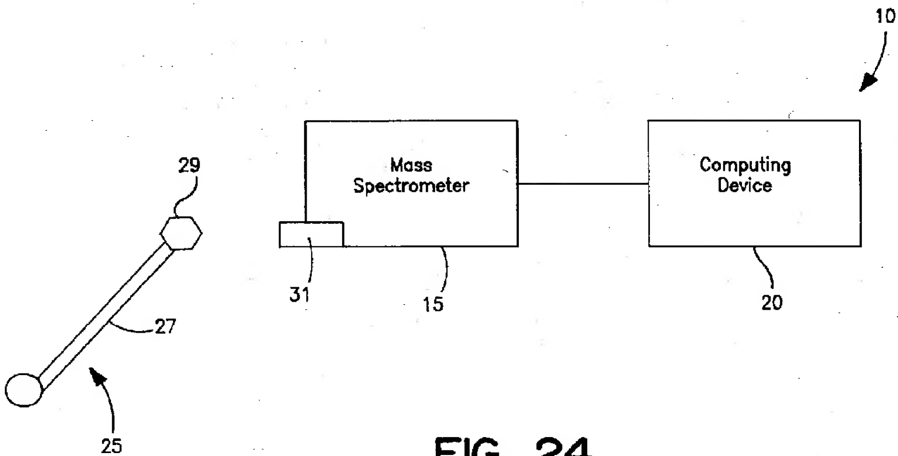


FIG. 24

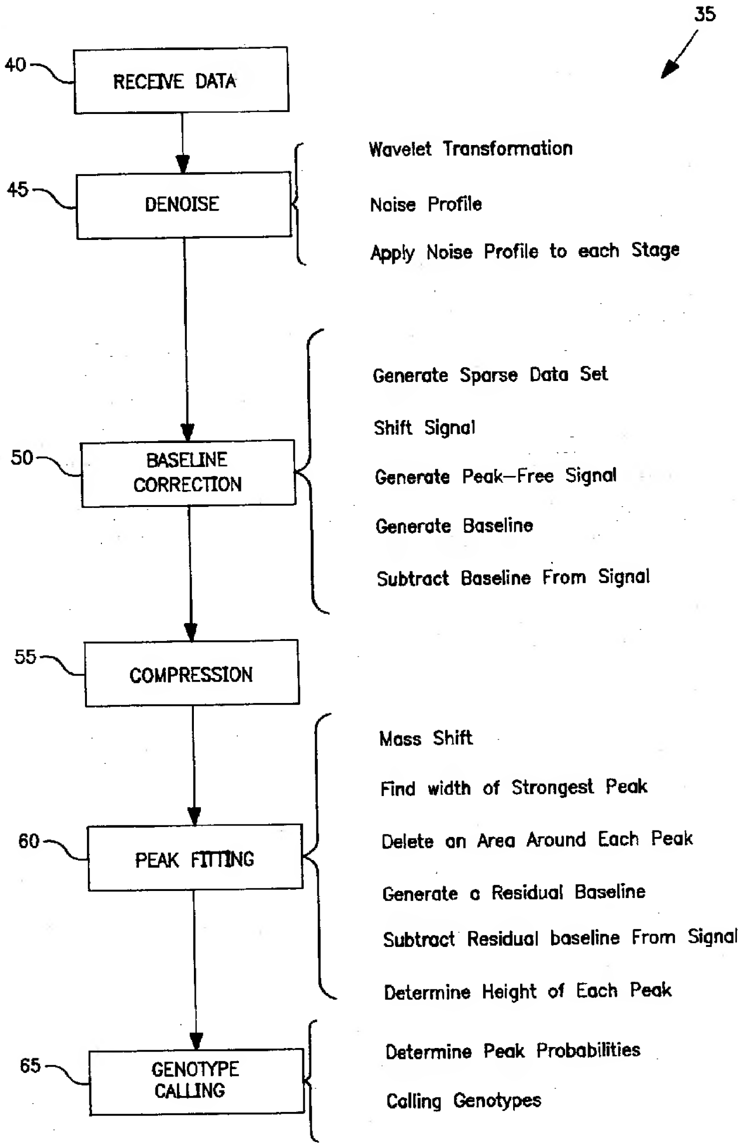

35
40
RECEIVE DATA
45
DENOISE
Wavelet Transformation
Noise Profile
Apply Noise Profile to each Stage
50
BASELINE CORRECTION
Generate Sparse Data Set
Shift Signal
Generate Peak—Free Signal
Generate Baseline
Subtract Baseline From Signal
55
COMPRESSION
60
PEAK FITTING
Mass Shift
Find width of Strongest Peak
Delete an Area Around Each Peak
Generate a Residual Baseline
Subtract Residual baseline From Signal
Determine Height of Each Peak
65
GENOTYPE CALLING
Determine Peak Probabilities
Calling Genotypes

FIG. 25

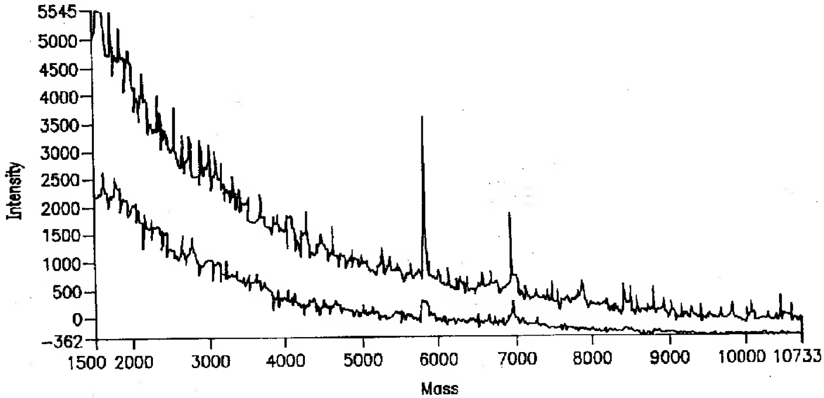

5545
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-362
Intensity
1500 2000 3000 4000 5000 6000 7000 8000 9000 10000 10733
Mass

FIG. 26

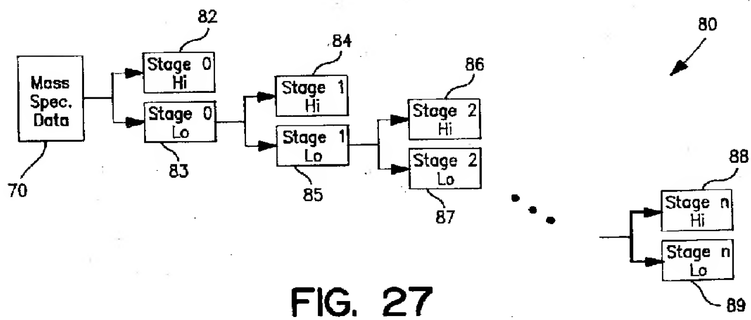

80
Mass Spec. Data
70
82
Stage 0 Hi
Stage 0 Lo
83
84
Stage 1 Hi
Stage 1 Lo
85
86
Stage 2 Hi
Stage 2 Lo
87
88
Stage n Hi
Stage n Lo
89

FIG. 27

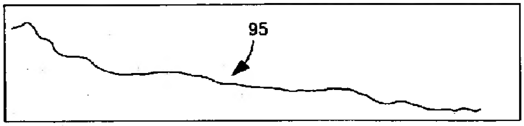


FIG. 28

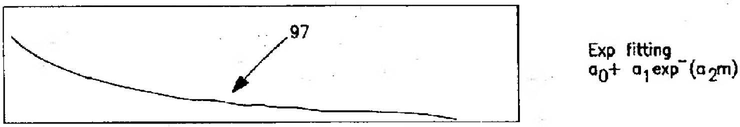


FIG. 29

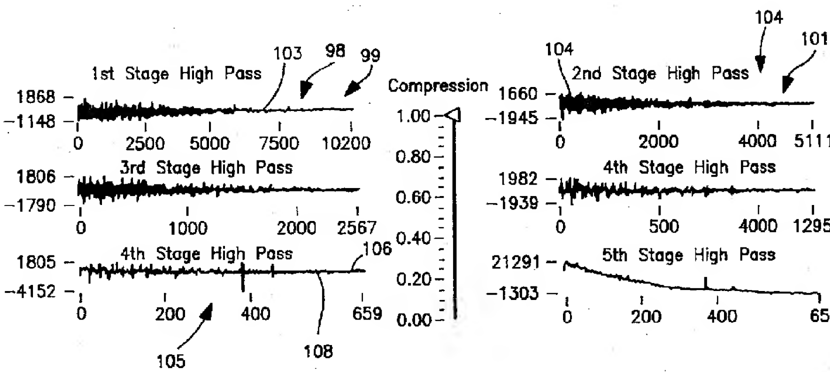


FIG. 30

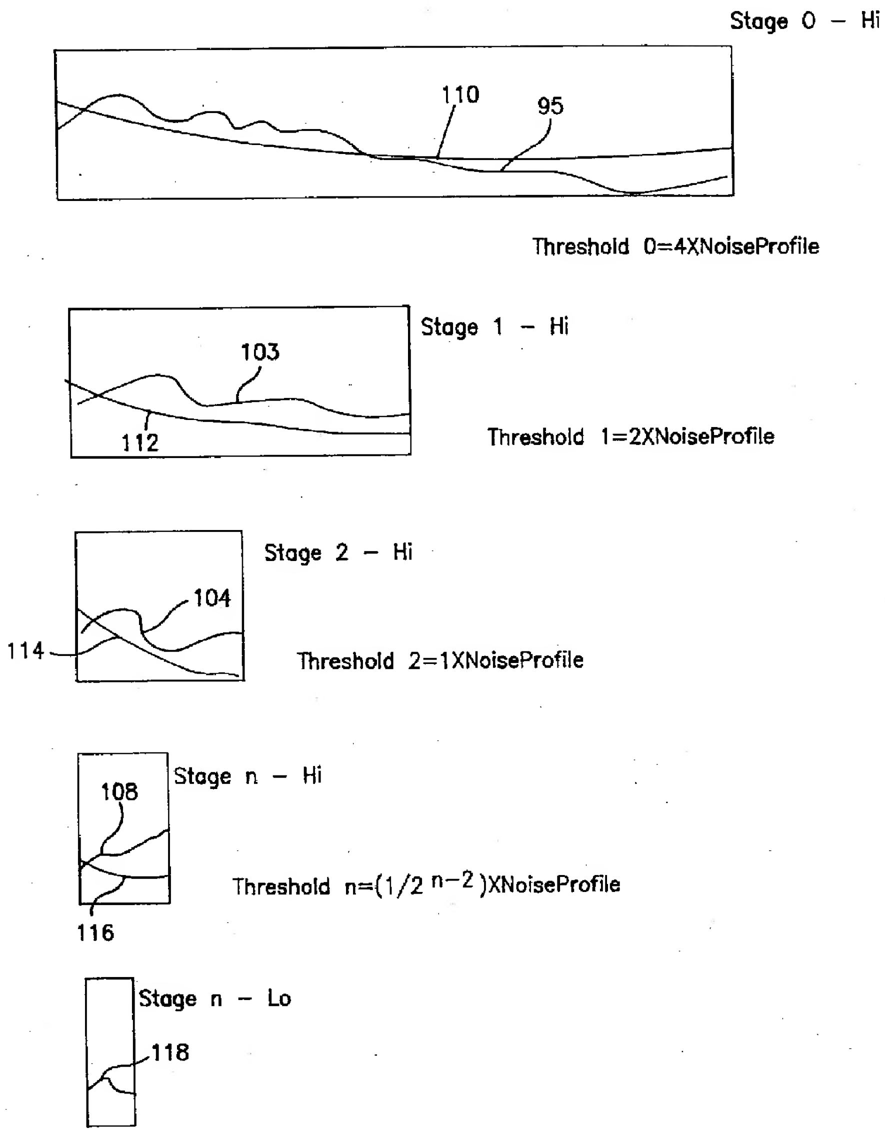

Stage 0 – Hi
110
95
Threshold 0=4XNoiseProfile
Stage 1 – Hi
103
112
Threshold 1=2XNoiseProfile
Stage 2 – Hi
104
114
Threshold 2=1XNoiseProfile
Stage n – Hi
108
116
Threshold n=(1/2 n−2)XNoiseProfile
Stage n – Lo
118

FIG. 31

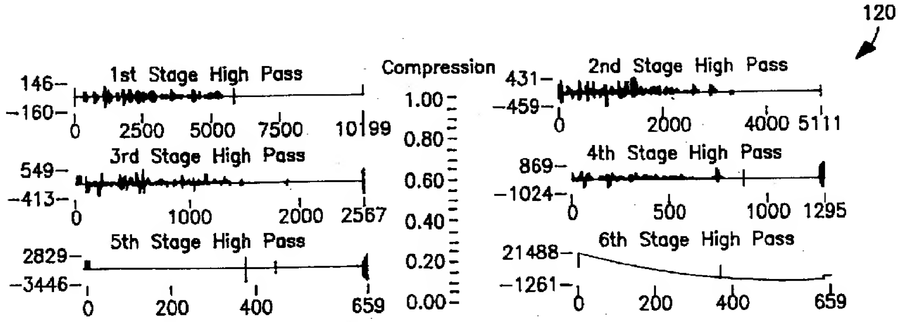


FIG. 32

$$\text{Signal }(t) = \frac{(\text{Start }0(t) + \text{Start }1(t) + \text{Start }2(t)\ldots + \text{Start }23\ (t))}{24}$$

SHIFT SIGNAL TO ACCOUNT FOR VARIATIONS DUE TO STARTING POINT

FIG. 33

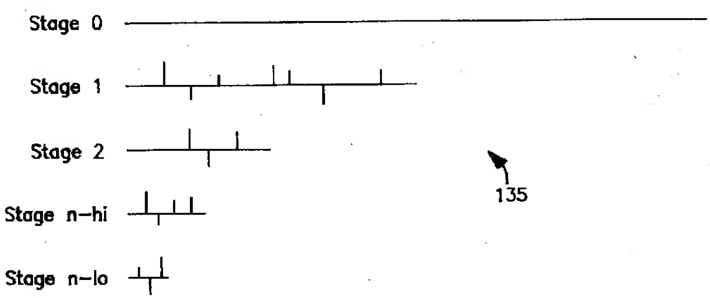


FIG. 34

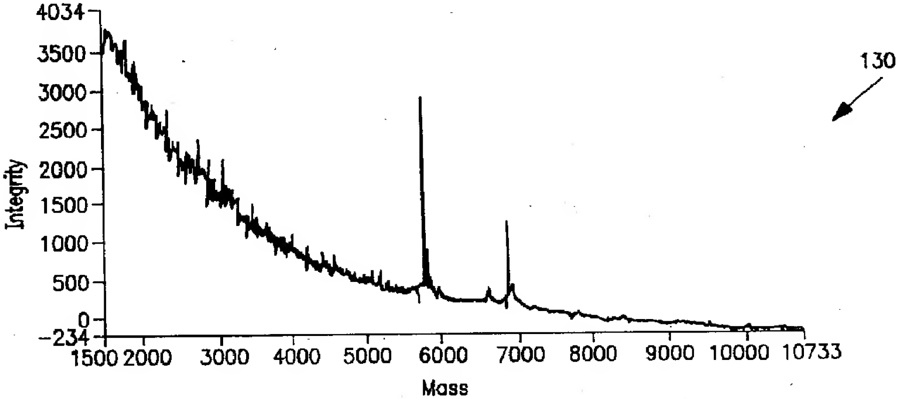


FIG. 35

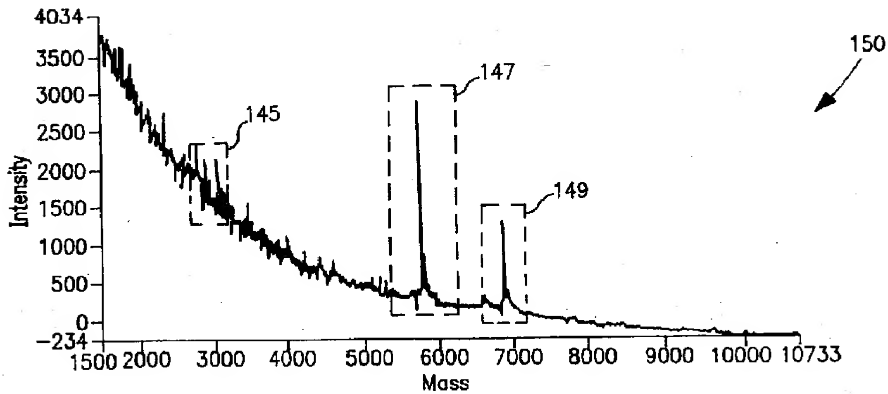


FIG. 13-TAKE A MOVING AVERAGE, REMOVE SECTIONS EXCEEDING A THRESHOLD

FIG. 36

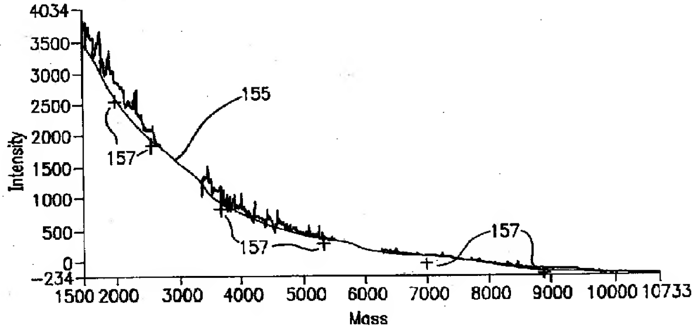


FIG. 37

FIND MINIMA IN REMAINING SIGNALS AND CONNECT TO FORM A PEAK FREE SIGNAL

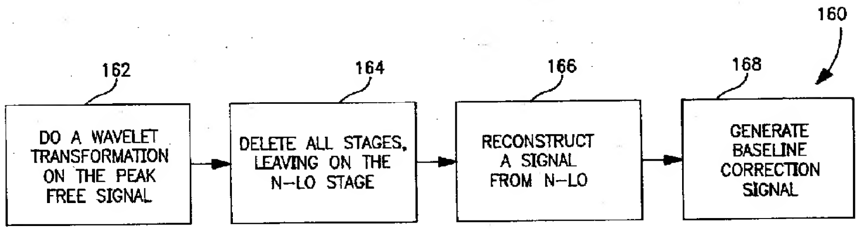


FIG. 38

GENRATE BASLELINE CORRECTION

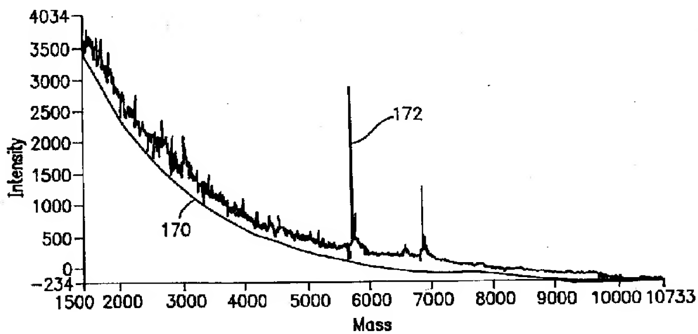


FIG. 39

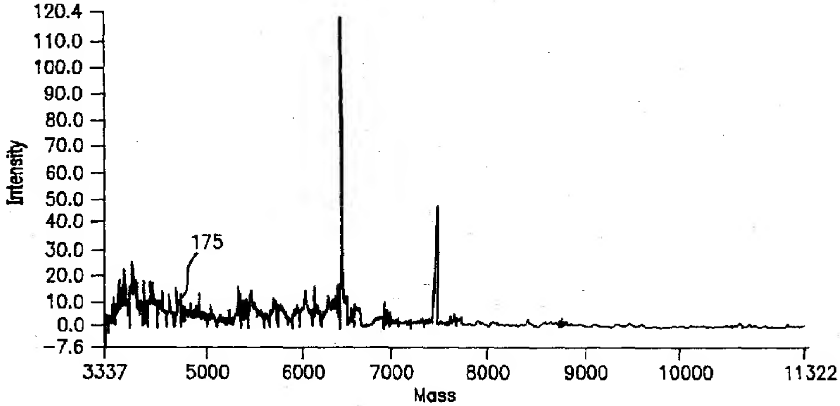


FIG. 40

180 182 183 184 186 188 190 195

NON-0 COEFFICIENTS	COEFFICIENTS	INTERMEDIATE	RELATIVE
100	25	100.025	100.025
150	220	150.220	50.220
500	.1	500.0001	350.0001
10,050	800	10,050.8	9550.8
10,075	890	10,075.89	25.89
11,125	910	11,125.91	150.91
12,100	1000 (MAX)	12,100.99999	975.99999
13,250	940	13,250.94	1150.94

FIG. 41

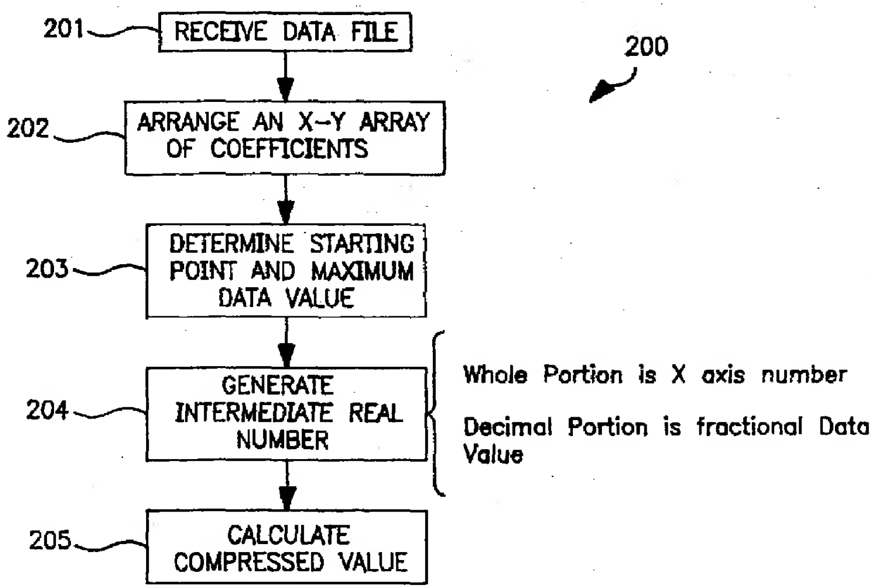

201
RECEIVE DATA FILE
200
202
ARRANGE AN X-Y ARRAY OF COEFFICIENTS
203
DETERMINE STARTING POINT AND MAXIMUM DATA VALUE
204
GENERATE INTERMEDIATE REAL NUMBER
Whole Portion is X axis number
Decimal Portion is fractional Data Value
205
CALCULATE COMPRESSED VALUE

FIG. 42

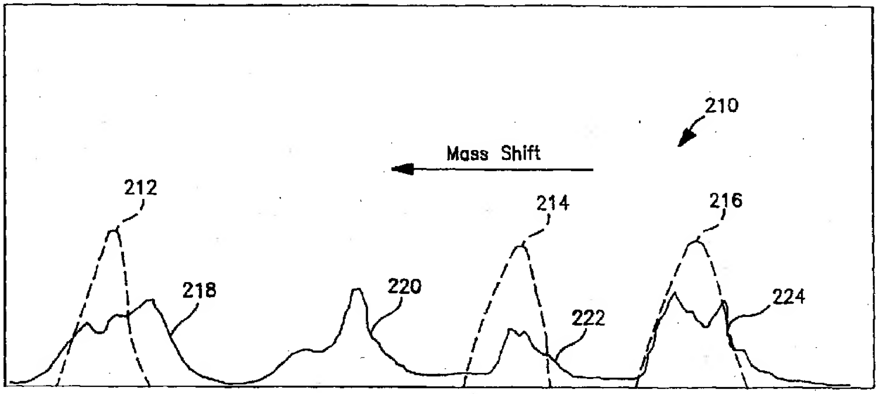


FIG. 43

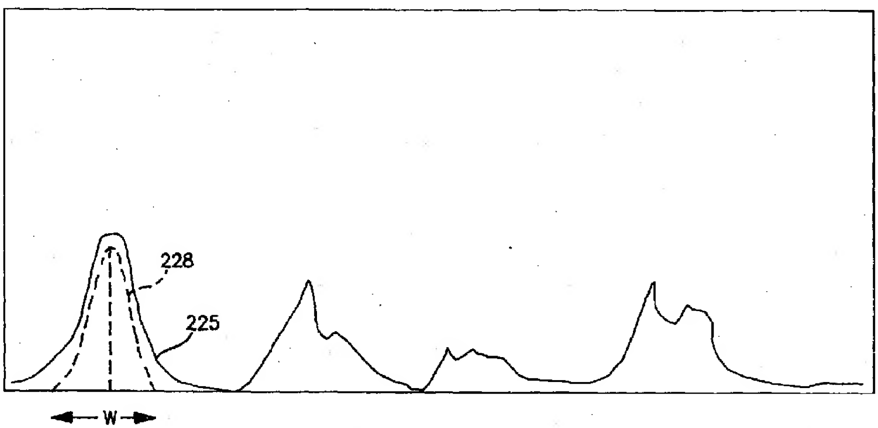


FIG. 44

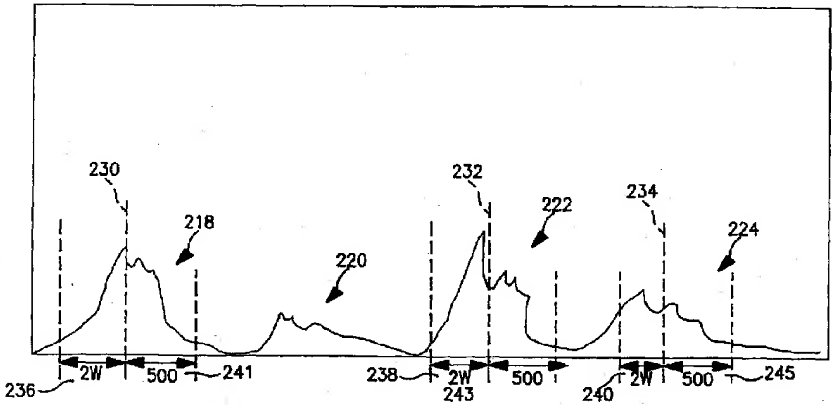


FIG. 45

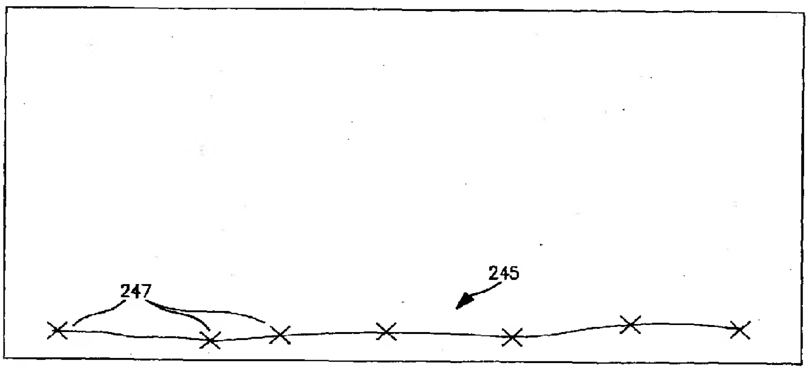


FIG. 46

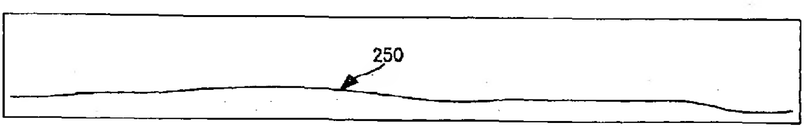


FIG. 47

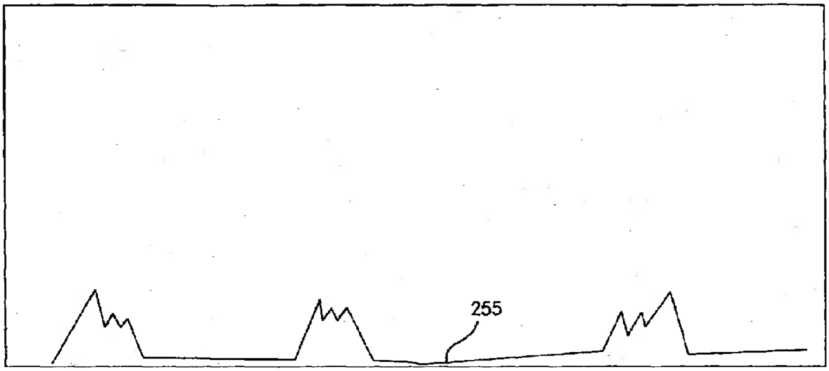


FIG. 48

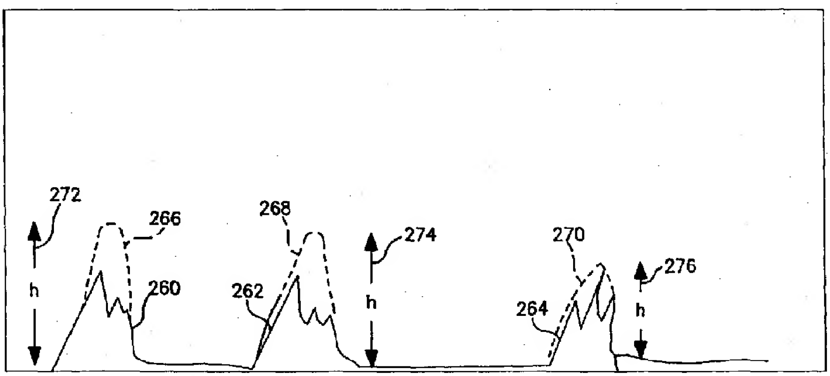


FIG. 49

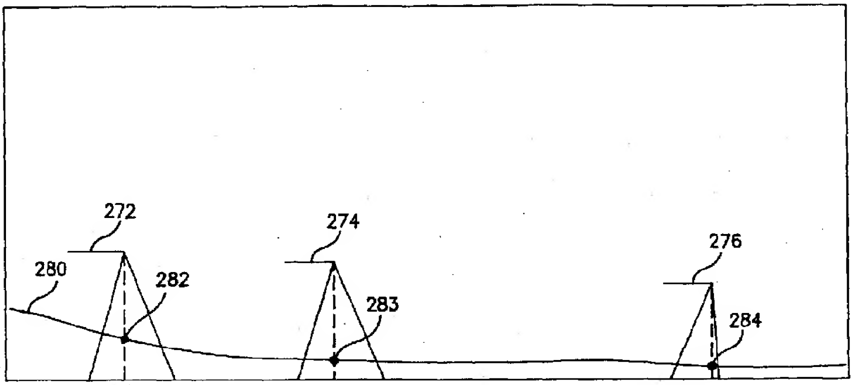


FIG. 50

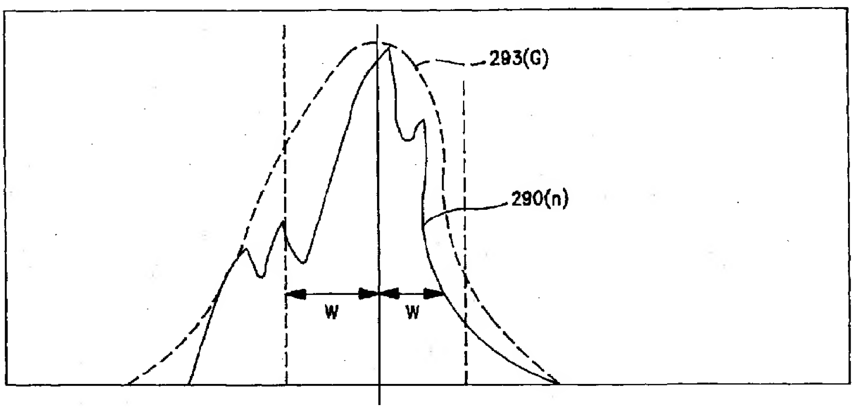


FIG. 51

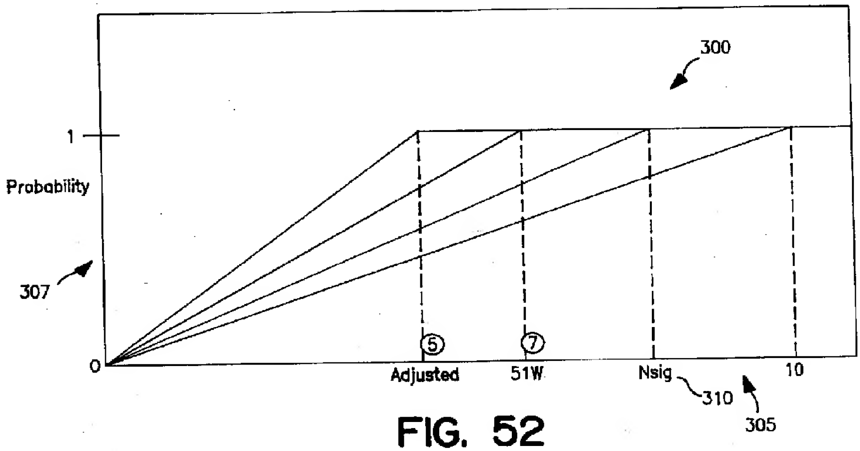

300
1
Probability
307
0
5
7
Adjusted
51W
Nsig
310
10
305

FIG. 52

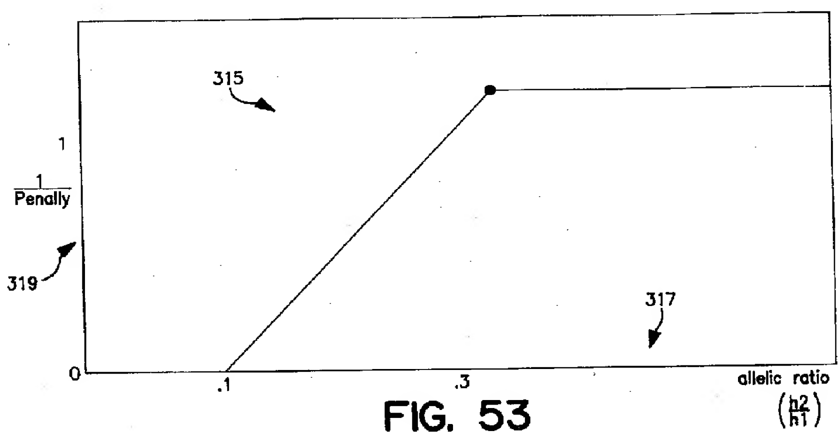
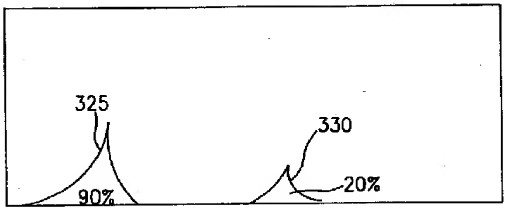

315
1
1
Penally
319
317
0
.1
.3
allelic ratio
(h2/h1)

FIG. 53

329 PROBABILTY OF GG EXISTING:

$$P(GG) = P(G) * P(1-C) = 90\% * (100\% - 20\%) = 90\% * 80\% = 72\%$$



331 PROBABILTY OF CC EXISTING:

$$P(CC) = P(C) * P(1-G) = 20\% * (100\% - 90\%) = 20\% * 10\% = 2\%$$

333 PROBABILTY OF GC EXISTING:

$$P(GC) = P(G) * P(C) = 90\% * 20\% = 18\%$$

FIG. 54

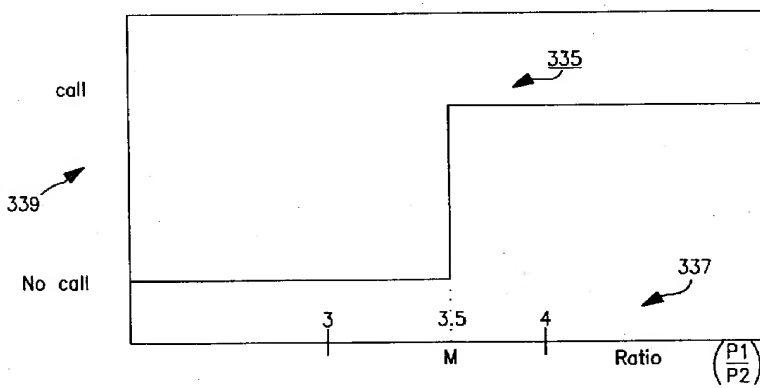


FIG. 55

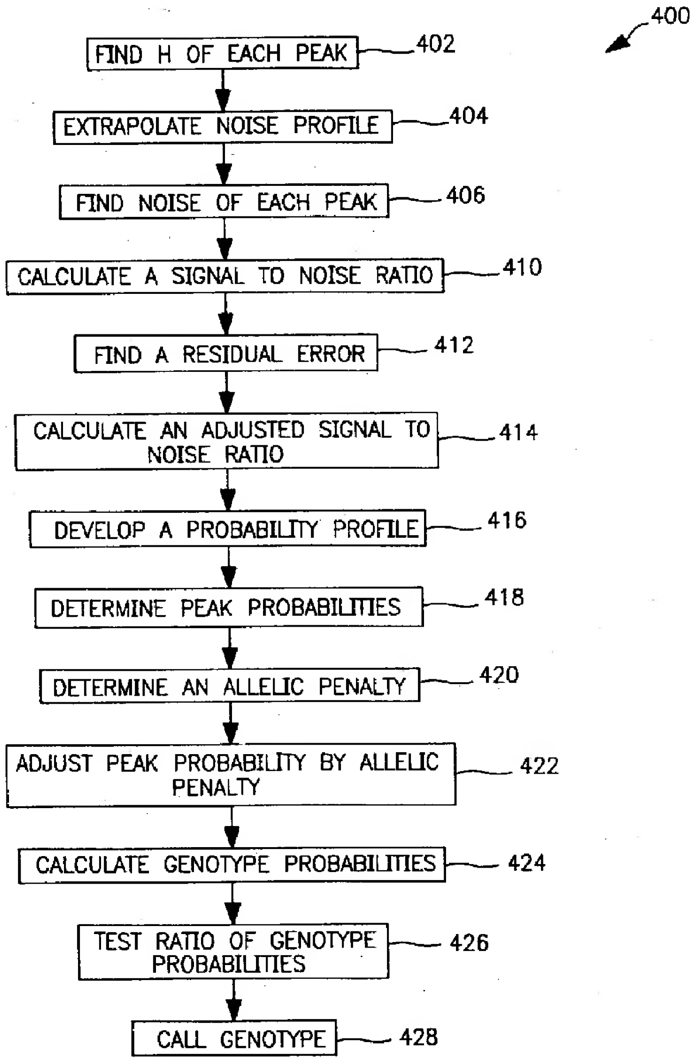

400
FIND H OF EACH PEAK
402
EXTRAPOLATE NOISE PROFILE
404
FIND NOISE OF EACH PEAK
406
CALCULATE A SIGNAL TO NOISE RATIO
410
FIND A RESIDUAL ERROR
412
CALCULATE AN ADJUSTED SIGNAL TO NOISE RATIO
414
DEVELOP A PROBABILITY PROFILE
416
DETERMINE PEAK PROBABILITIES
418
DETERMINE AN ALLELIC PENALTY
420
ADJUST PEAK PROBABILITY BY ALLELIC PENALTY
422
CALCULATE GENOTYPE PROBABILITIES
424
TEST RATIO OF GENOTYPE PROBABILITIES
426
CALL GENOTYPE
428

FIG. 56

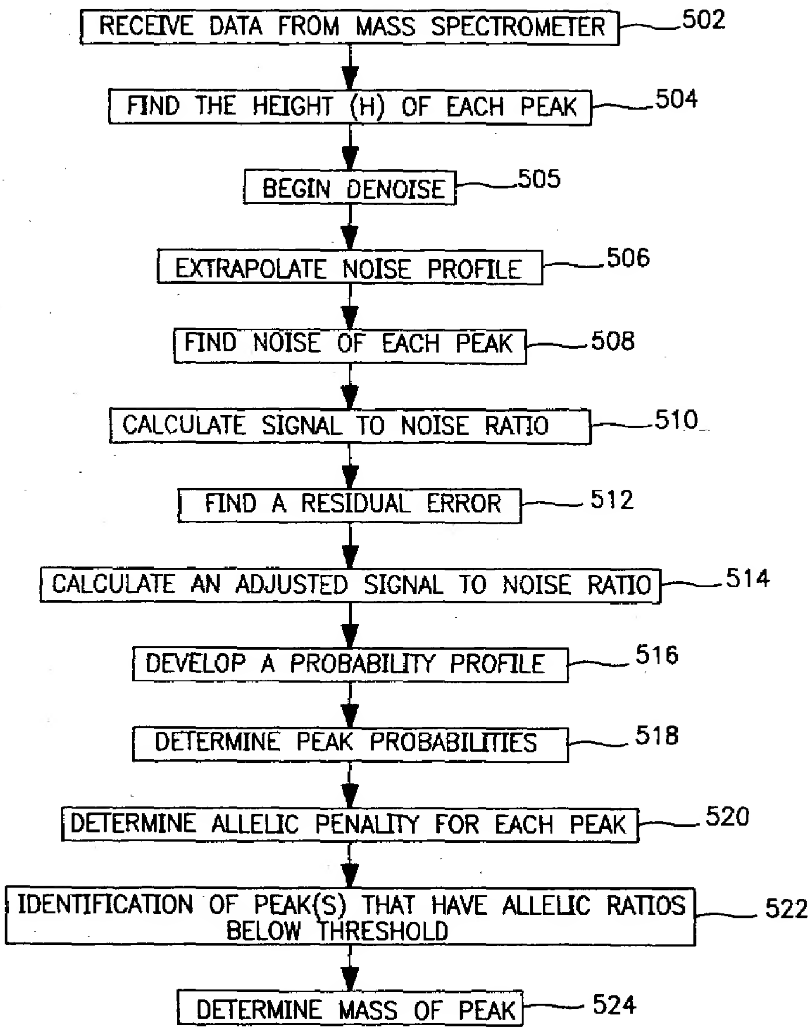

RECEIVE DATA FROM MASS SPECTROMETER
502
FIND THE HEIGHT (H) OF EACH PEAK
504
BEGIN DENOISE
505
EXTRAPOLATE NOISE PROFILE
506
FIND NOISE OF EACH PEAK
508
CALCULATE SIGNAL TO NOISE RATIO
510
FIND A RESIDUAL ERROR
512
CALCULATE AN ADJUSTED SIGNAL TO NOISE RATIO
514
DEVELOP A PROBABILITY PROFILE
516
DETERMINE PEAK PROBABILITIES
518
DETERMINE ALLELIC PENALITY FOR EACH PEAK
520
IDENTIFICATION OF PEAK(S) THAT HAVE ALLELIC RATIOS BELOW THRESHOLD
522
DETERMINE MASS OF PEAK
524

FIG. 57

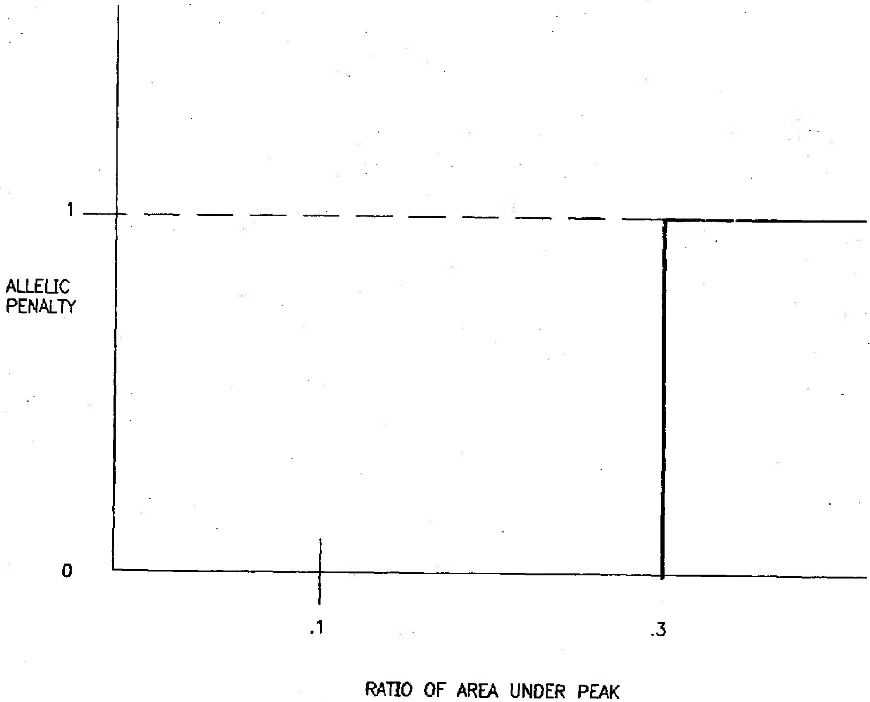


FIG. 58

METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS

RELATED APPLICATIONS

[0001] This application is a divisional application of copending U.S. patent application Ser. No. 09/687,483, filed Oct. 13, 2000, to Andreas Braun, Hubert Koster, Dirk Van den Boom, Yip Ping, Charles Rodi, Liyan He, Norman Chiu and Christian Jurinke entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS."

[0002] Benefit of priority under 35 U.S.C. §119(e) to the following provisional applications is claimed herein: U.S. provisional application Serial No. 60/217,658 to Andreas Braun, Hubert Koster; Dirk Van den Boom, filed Jul. 10, 2000, entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS"; U.S. provisional application Serial No. 60/159,176 to Andreas Braun, Hubert Koster, Dirk Van den Boom, filed Oct. 13, 1999, entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING POLYMORPHIC GENETIC MARKERS"; U.S. provisional application Serial No. 60/217,251, filed Jul. 10, 2000, to Andreas Braun, entitled "POLYMORPHIC KINASE ANCHOR PROTEIN GENE SEQUENCES, POLYMORPHIC KINASE ANCHOR PROTEINS AND METHODS OF DETECTING POLYMORPHIC KINASE ANCHOR PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME". This application is also a continuation-in-part of U.S. application Serial No. 09/663,968, to Ping Yip, filed Sep. 19, 2000, entitled "METHOD AND DEVICE FOR IDENTIFYING A BIOLOGICAL SAMPLE."

[0003] The above-noted applications and provisional applications are incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0004] Process and methods for creating a database of genomic samples from healthy human donors. Methods that use the database to identify and correlate with polymorphic genetic markers and other markers with diseases and conditions are provided.

BACKGROUND

[0005] Diseases in all organisms have a genetic component, whether inherited or resulting from the body's response to environmental stresses, such as viruses and toxins. The ultimate goal of ongoing genomic research is to use this information to develop new ways to identify, treat and potentially cure these diseases. The first step has been to screen disease tissue and identify genomic changes at the level of individual samples. The identification of these "disease" markers has then fueled the development and commercialization of diagnostic tests that detect these errant genes or polymorphisms. With the increasing numbers of genetic markers, including single nucleotide polymorphisms (SNPs), microsatellites, tandem repeats, newly mapped introns and exons, the challenge to the medical and pharmaceutical communities is to identify genotypes which not only identify the disease but also follow the progression of the disease and are predictive of an organism's response to treatment.

[0006] Currently the pharmaceutical and biotechnology industries find a disease and then attempt to determine the genomic basis for the disease. This approach is time consuming and expensive and in many cases involves the investigator guessing as to what pathways might be involved in the disease.

[0007] Genomics

[0008] Presently the two main strategies employed in analyzing the available genomic information are the technology driven reverse genetics brute force strategy and the knowledge-based pathway oriented forward genetics strategy. The brute force approach yields large databases of sequence information but little information about the medical or other uses of the sequence information. Hence this strategy yields intangible products of questionable value. The knowledge-based strategy yields small databases that contain a lot of information about medical uses of particular DNA sequences and other products in the pathway and yield tangible products with a high value.

[0009] Polymorphisms

[0010] Polymorphisms have been known since 1901 with the identification of blood types. In the 1950's they were identified on the level of proteins using large population genetic studies. In the 1980's and 1990's many of the known protein polymorphisms were correlated with genetic loci on genomic DNA. For example, the gene dose of the apolipoprotein E type 4 allele was correlated with the risk of Alzheimer's disease in late onset families (see, e.g., Corder et al. (1993) *Science* 261: 921-923; mutation in blood coagulation factor V was associated with resistance to activated protein C (see, e.g., Bertina et al. (1994) *Nature* 369:64-67); resistance to HIV-1 infection has been shown in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene (see, e.g., Samson et al. (1996) *Nature* 382:722-725); and a hypermutable tract in antigen presenting cells (APC, such as macrophages), has been identified in familial colorectal cancer in individuals of Ashkenzi jewish background (see, e.g., Laken et al. (1997) *Nature Genet.* 17:79-83). There can be more than three million polymorphic sites in the human genome. Many have been identified, but not yet characterized or mapped or associated with a marker.

[0011] Single Nucleotide Polymorphisms (SNPs)

[0012] Much of the focus of genomics has been in the identification of SNPs, which are important for a variety of reasons. They allow indirect testing (association of haplotypes) and direct testing (functional variants). They are the most abundant and stable genetic markers. Common diseases are best explained by common genetic alterations, and the natural variation in the human population aids in understanding disease, therapy and environmental interactions.

[0013] Currently, the only available method to identify SNPs in DNA is by sequencing, which is expensive, difficult and laborious. Furthermore, once a SNP is discovered it must be validated to determine if it is a real polymorphism and not a sequencing error. Also, discovered SNPs must then be evaluated to determine if they are associated with a particular phenotype. Thus, there is a need to develop new paradigms for identifying the genomic basis for disease and markers thereof. Therefore, it is an object herein to provide methods for identifying the genomic basis of disease and markers thereof.

SUMMARY

[0014] Databases and methods using the databases are provided herein. The databases comprise sets of parameters associated with subjects in populations selected only on the basis of being healthy (i.e., where the subjects are mammals, such as humans, they are selected based upon apparent health and no detectable infections). The databases can be sorted based upon one or more of the selected parameters.

[0015] The databases, for example, can be relational databases, in which an index that represents each subject serves to relate parameters, which are the data, such as age, ethnicity, sex, medical history, etc. and ultimately genotypic information, that was inputted into and stored in the database. The database can then be sorted according to these parameters. Initially, the parameter information is obtained from a questionnaire answered by each subject from whom a body tissue or body fluid sample is obtained. As additional information about each sample is obtained, this information can be entered into the database and can serve as a sorting parameter.

[0016] The databases obtained from healthy individuals have numerous uses, such as correlating known polymorphisms with a phenotype or disease. The databases can be used to identify alleles that are deleterious, that are beneficial, and that are correlated with diseases.

[0017] For purposes herein, genotypic information can be obtained by any method known to those of skill in the art, but is generally obtained using mass spectrometry.

[0018] Also provided herein, is a new use for existing databases of subjects and genotypic and other parameters, such as age, ethnicity, race, and gender. Any database can be sorted according to the methods herein, and alleles that exhibit statistically significant correlations with any of the sorting parameters can be identified. It is noted, however, is noted, that the databases provided herein and randomly selected databases will perform better in these methods, since disease-based databases suffer numerous limitations, including their relatively small size, the homogeneity of the selected disease population, and the masking effect of the polymorphism associated with the markers for which the database was selected. Hence, the healthy database provided herein, provides advantages not heretofore recognized or exploited. The methods provided herein can be used with a selected database, including disease-based databases, with or without sorting for the discovery and correlation of polymorphisms. In addition, the databases provided herein represent a greater genetic diversity than the unselected databases typically utilized for the discovery of polymorphisms and thus allow for the enhanced discovery and correlation of polymorphisms.

[0019] The databases provided herein can be used for taking an identified polymorphism and ascertaining whether it changes in frequency when the data are sorted according to a selected parameter.

[0020] One use of these methods is correlating a selected marker with a particular parameter by following the occurrence of known genetic markers and then, having made this correlation, determining or identifying correlations with diseases. Examples of this use are p53 and Lipoprotein Lipase polymorphism. As exemplified herein, known markers are shown to have particular correlation with certain groups, such as a particular ethnicity or race or one sex. Such correlations will then permit development of better diagnostic tests and treatment regimens.

[0021] These methods are valuable for identifying one or more genetic markers whose frequency changes within the population as a function of age, ethnic group, sex or some other criteria. This can allow the identification of previously unknown polymorphisms and ultimately a gene or pathway involved in the onset and progression of disease.

[0022] The databases and methods provided herein permit, among other things, identification of components, particularly key components, of a disease process by understanding its genetic underpinnings and also permit an understanding of processes, such as individual drug responses. The databases and methods provided herein also can be used in methods involving elucidation of pathological pathways, in developing new diagnostic assays, identifying new potential drug targets, and in identifying new drug candidates.

[0023] The methods and databases can be used with experimental procedures, including, but are not limited to, in silico SNP identification, in vitro SNP identification/verification, genetic profiling of large populations, and in biostatistical analyses and interpretations.

[0024] Also provided herein, are combinations that contain a database provided herein and a biological sample from a subject in the database, and typically biological samples from all subjects or a plurality of subjects in the database. Collections of the tissue and body fluid samples are also provided.

[0025] Also, provided herein, are methods for determining a genetic marker that correlates with age, comprising identifying a polymorphism and determining the frequency of the polymorphism with increasing age in a healthy population.

[0026] Further provided herein are methods for determining whether a genetic marker correlates with susceptibility to morbidity, early mortality, or morbidity and early mortality, comprising identifying a polymorphism and determining the frequency of the polymorphism with increasing age in a healthy population.

[0027] Any of the methods herein described can be used out in a multiplex format.

[0028] Also provided are an apparatus and process for accurately identifying genetic information. It is another object herein that genetic information be extracted from genetic data in a highly automated manner. Therefore, to overcome the deficiencies in the known conventional systems, methods and apparatus for identifying a biological sample are provided.

[0029] Briefly, the method and system for identifying a biological sample generates a data set indicative of the composition of the biological sample. In a particular example, the data set is DNA spectrometry data received from a mass spectrometer. The data set is denoised, and a baseline is deleted. Since possible compositions of the biological sample can be known, expected peak areas can be determined. Using the expected peak areas, a residual baseline is generated to further correct the data set. Probable peaks are then identifiable in the corrected data set, which are used to identify the composition of the biological

sample. In a disclosed example, statistical methods are employed to determine the probability that a probable peak is an actual peak, not an actual peak, or that the data too inconclusive to call.

[0030] Advantageously, the method and system for identifying a biological sample accurately makes composition calls in a highly automated manner. In such a manner, complete SNP profile information, for example, can be collected efficiently. More importantly, the collected data are analyzed with highly accurate results. For example, when a particular composition is called, the result can be relied upon with great confidence. Such confidence is provided by the robust computational process employed.

DESCRIPTION OF THE DRAWINGS

[0031] **FIG. 1** depicts an exemplary sample bank. Panel 1 shows the samples as a function of sex and ethnicity. Panel 2 shows the Caucasians as a function of age. Panel 3 shows the Hispanics as a function of age.

[0032] **FIGS. 2A and 2C** show an age- and sex-distribution of the 291S allele of the lipoprotein lipase gene in which a total of 436 males and 589 females were investigated. **FIG. 2B** shows an age distribution for the 436 males.

[0033] **FIG. 3** is an exemplary questionnaire for population-based sample banking.

[0034] **FIG. 4** depicts processing and tracking of blood sample components.

[0035] **FIG. 5** depicts the allelic frequency of "sick" alleles and "healthy" alleles as a function of age. It is noted that the relative frequency of healthy alleles increases in a population with increasing age.

[0036] **FIG. 6** depicts the age-dependent distribution of ApoE genotypes (see, Schächter et al. (1994) *Nature Genetics* 6:29-32).

[0037] FIGS. 7A-D depicts age-related and genotype frequency of the p53 (tumor suppressor) codon 72 among the Caucasian population in the database. *R72 and *P72 represent the frequency of the allele in the database population. R72, R72P, and P72 represent the genotypes of the individuals in the population. The frequency of the homozygous P72 allele drops from 6.7% to 3.7% with age.

[0038] **FIG. 8** depicts the allele and genotype frequencies of the p21 S31R allele as a function of age.

[0039] **FIG. 9** depicts the frequency of the FVII Allele 353Q in pooled versus individual samples.

[0040] **FIG. 10** depicts the frequency of the CETP (cholesterol ester transfer protein) allele in pooled versus individual samples.

[0041] **FIG. 11** depicts the frequency of the plasminogen activator inhibitor-1 (PAI-1) 5G in pooled versus individual samples.

[0042] **FIG. 12** shows mass spectra of the samples and the ethnic diversity of the PAI-1 alleles.

[0043] **FIG. 13** shows mass spectra of the samples and the ethnic diversity of the CETP 405 alleles.

[0044] **FIG. 14** shows mass spectra of the samples and the ethnic diversity of the Factor VII 353 alleles.

[0045] **FIG. 15** shows ethnic diversity of PAI-1, CETP and Factor VII using the pooled DNA samples.

[0046] **FIG. 16** shows the p53-Rb pathway and the relationships among the various factors in the pathway.

[0047] **FIG. 17**, which is a block diagram of a computer constructed to provide and process the databases described herein, depicts a typical computer system for storing and sorting the databases provided herein and practicing the methods provided herein.

[0048] **FIG. 18** is a flow diagram that illustrates the processing steps performed using the computer illustrated in **FIG. 17**, to maintain and provide access to the databases for identifying polymorphic genetic markers.

[0049] **FIG. 19** is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the AKAP10-1 locus. Bright green bars show frequencies in individuals younger than 40 years. Dark green bars show frequencies in individuals older than 60 years.

[0050] **FIG. 20** is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the AKAP10-5 locus. Bright green bars show frequencies in individuals younger than 40 years; dark green bars show frequencies in individuals older than 60 years.

[0051] **FIG. 21** is a histogram showing the allele and genotype distribution in the age and sex stratified Caucasian population for the h-msrA locus. Genotype difference between male age groups is significant. Bright green bars show frequencies in individuals younger than 40 years. Dark green bars show frequencies in individuals older than 60 years.

[0052] FIGS. 22A-D is a sample data collection questionnaire used for the healthy database.

[0053] **FIG. 23** is a flowchart showing processing performed by the computing device of **FIG. 24** when performing genotyping of sense strands and antisense strands from assay fragments.

[0054] **FIG. 24** is a block diagram showing a system provided herein;

[0055] **FIG. 25** is a flowchart of a method of identifying a biological sample provided herein;

[0056] **FIG. 26** is a graphical representation of data from a mass spectrometer;

[0057] **FIG. 27** is a diagram of wavelet transformation of mass spectrometry data;

[0058] **FIG. 28** is a graphical representation of wavelet stage 0 hi data;

[0059] **FIG. 29** is a graphical representation of stage 0 noise profile;

[0060] **FIG. 30** is a graphical representation of generating stage noise standard deviations;

[0061] **FIG. 31** is a graphical representation of applying a threshold to data stages;

[0062] **FIG. 32** is a graphical representation of a sparse data set;

[0063] **FIG. 33** is a formula for signal shifting;

[0064] **FIG. 34** is a graphical representation of a wavelet transformation of a denoised and shifted signal;

[0065] **FIG. 35** is a graphical representation of a denoised and shifted signal;

[0066] **FIG. 36** is a graphical representation of removing peak sections;

[0067] **FIG. 37** is a graphical representation of generating a peak free signal;

[0068] **FIG. 38** is a block diagram of a method of generating a baseline correction;

[0069] **FIG. 39** is a graphical representation of a baseline and signal;

[0070] **FIG. 40** is a graphical representation of a signal with baseline removed;

[0071] **FIG. 41** is a table showing compressed data;

[0072] **FIG. 42** is a flowchart of method for compressing data;

[0073] **FIG. 43** is a graphical representation of mass shifting;

[0074] **FIG. 44** is a graphical representation of determining peak width;

[0075] **FIG. 45** is a graphical representation of removing peaks;

[0076] **FIG. 46** is a graphical representation of a signal with peaks removed;

[0077] **FIG. 47** is a graphical representation of a residual baseline;

[0078] **FIG. 48** is a graphical representation of a signal with residual baseline removed;

[0079] **FIG. 49** is a graphical representation of determining peak height;

[0080] **FIG. 50** is a graphical representation of determining signal-to-noise for each peak;

[0081] **FIG. 51** is a graphical representation of determining a residual error for each peak;

[0082] **FIG. 52** is a graphical representation of peak probabilities;

[0083] **FIG. 53** is a graphical representation of applying an allelic ratio to peak probability;

[0084] **FIG. 54** is a graphical representation of determining peak probability;

[0085] **FIG. 55** is a graphical representation of calling a genotype;

[0086] **FIG. 56** is a flowchart showing a statistical procedure for calling a genotype;

[0087] **FIG. 57** is a flowchart showing processing performed by the computing device of **FIG. 1** when performing standardless genotyping; and

[0088] **FIG. 58** is graphical representation of applying an allelic ratio to peak probability for standardless genotype processing.

DETAILED DESCRIPTION

[0089] Definitions

[0090] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications and sequences from GenBank and other databases referred to herein throughout the disclosure are incorporated by reference in their entirety.

[0091] As used herein, a biopolymer includes, but is not limited to, nucleic acid, proteins, polysaccharides, lipids and other macromolecules. Nucleic acids include DNA, RNA, and fragments thereof. Nucleic acids can be derived from genomic DNA, RNA, mitochondrial nucleic acid, chloroplast nucleic acid and other organelles with separate genetic material.

[0092] As used herein, morbidity refers to conditions, such as diseases or disorders, that compromise the health and well-being of an organism, such as an animal. Morbidity susceptibility or morbidity-associated genes are genes that, when altered, for example, by a variation in nucleotide sequence, facilitate the expression of a specific disease clinical phenotype. Thus, morbidity susceptibility genes have the potential, upon alteration, of increasing the likelihood or general risk that an organism will develop a specific disease.

[0093] As used herein, mortality refers to the statistical likelihood that an organism, particularly an animal, will not survive a full predicted lifespan. Hence, a trait or a marker, such as a polymorphism, associated with increased mortality is observed at a lower frequency in older than younger segments of a population.

[0094] As used herein, a polymorphism, e.g. genetic variation, refers to a variation in the sequence of a gene in the genome amongst a population, such as allelic variations and other variations that arise or are observed. Thus, a polymorphism refers to the occurrence of two or more genetically determined alternative sequences or alleles in a population. These differences can occur in coding and non-coding portions of the genome, and can be manifested or detected as differences in nucleic acid sequences, gene expression, including, for example transcription, processing, translation, transport, protein processing, trafficking, DNA synthesis, expressed proteins, other gene products or products of biochemical pathways or in post-translational modifications and any other differences manifested amongst members of a population. A single nucleotide polymorphism (SNP) refers to a polymorphism that arises as the result of a single base change, such as an insertion, deletion or change in a base.

[0095] A polymorphic marker or site is the locus at which divergence occurs. Such site can be as small as one base pair (an SNP). Polymorphic markers include, but are not limited to, restriction fragment length polymorphisms, variable number of tandem repeats (VNTR's), hypervariable regions, minisatellites, dinucleotide repeats, trinucleotide repeats, tetranucleotide repeats and other repeating patterns, simple sequence repeats and insertional elements, such as Alu. Polymorphic forms also are manifested as different mendelian alleles for a gene. Polymorphisms can be observed by differences in proteins, protein modifications, RNA expression modification, DNA and RNA methylation, regulatory

factors that alter gene expression and DNA replication, and any other manifestation of alterations in genomic nucleic acid or organelle nucleic acids.

[0096] As used herein, a healthy population refers to a population of organisms, including but are not limited to, animals, bacteria, viruses, parasites, plants, eubacteria, and others, that are disease free. The concept of disease-free is a function of the selected organism. For example, for mammals it refers to a subject not manifesting any disease state. Practically a healthy subject, when human, is defined as human donor who passes blood bank criteria to donate blood for eventual use in the general population. These criteria are as follows: free of detectable viral, bacterial, mycoplasma, and parasitic infections; not anemic; and then further selected based upon a questionnaire regarding history (see **FIG. 3**). Thus, a healthy population represents an unbiased population of sufficient health to donate blood according to blood bank criteria, and not further selected for any disease state. Typically such individuals are not taking any medications. For plants, for example, it is a plant population that does not manifest diseases pathology associated with plants. For bacteria it is a bacterial population replicating without environmental stress, such as selective agents, heat and other pathogens.

[0097] As used herein, a healthy database (or healthy patient database) refers to a database of profiles of subjects that have not been pre-selected for any particular disease. Hence, the subjects that serve as the source of data for the database are selected, according to predetermined criteria, to be healthy. In contrast to other such databases that have been pre-selected for subjects with a particular disease or other characteristic, the subjects for the database provided herein are not so-selected. Also, if the subjects do manifest a disease or other condition, any polymorphism discovered or characterized should be related to an independent disease or condition. In a one embodiment, where the subjects are human, a healthy subject manifests no disease symptoms and meets criteria, such as those set by blood banks for blood donors.

[0098] Thus, the subjects for the database are a population of any organism, including, but are not limited to, animals, plants, bacteria, viruses, parasites and any other organism or entity that has nucleic acid. Among subjects are mammals, such as, although not necessarily, humans. Such a database can capture the diversity of a population, thus providing for discovery of rare polymorphisms.

[0099] As used herein, a profile refers to information relating to, but not limited to and not necessarily including all of, age, sex, ethnicity, disease history, family history, phenotypic characteristics, such as height and weight and other relevant parameters. A sample collect information form is shown in **FIG. 22**, which illustrates profile intent.

[0100] As used herein, a disease state is a condition or abnormality or disorder that can be inherited or result from environmental stresses, such as toxins, bacterial, fungal and viral infections.

[0101] As used herein, set of non-selected subjects means that the subjects have not been pre-selected to share a common disease or other characteristic. They can be selected to be healthy as defined herein.

[0102] As used herein, a phenotype refers to a set of parameters that includes any distinguishable trait of an organism. A phenotype can be physical traits and can be, in instances in which the subject is an animal, a mental trait, such as emotional traits. Some phenotypes can be determined by observation elicited by questionnaires (see, e.g., **FIGS. 3 and 22**) or by referring to prior medical and other records. For purposes herein, a phenotype is a parameter around which the database can be sorted.

[0103] As used herein, a parameter is any input data that will serve as a basis for sorting the database. These parameters will include phenotypic traits, medical histories, family histories and any other such information elicited from a subject or observed about the subject. A parameter can describe the subject, some historical or current environmental or social influence experienced by the subject, or a condition or environmental influence on someone related to the subject. Paramaters include, but are not limited to, any of those described herein, and known to those of skill in the art.

[0104] As used herein, haplotype refers to two or polymorphism located on a single DNA strand. Hence, haplotyping refers to identification of two or more polymorphisms on a single DNA strand. Haplotypes can be indicative of a phenotype. For some disorders a single polymorphism can suffice to indicate a trait; for others a plurality (i.e., a haplotype) can be needed. Haplotyping can be performed by isolating nucleic acid and separating the strands. In addition, when using enzymes such a certain nucleases, that produce, different size fragments from each strand, strand separation is not needed for haplotyping.

[0105] As used herein, pattern with reference to a mass spectrum or mass spectrometric analyses, refers to a characteristic distribution and number of signals (such peaks or digital representations thereof).

[0106] As used herein, signal in the context of a mass spectrum and analysis thereof refers to the output data, which the number or relative number of moleucles having a particular mass. Signals include "peaks" and digital representations thereof.

[0107] As used herein, adaptor, when used with reference to haplotyping using Fen ligase, refers to a nucleic acid that specifically hybridizes to a polymorphism of interest. An adaptor can be partially double-stranded. An adaptor complex is formed when an adaptor hybridizes to its target.

[0108] As used herein, a target nucleic acid refers to any nucleic acid of interest in a sample. It can contain one or more nucleotides.

[0109] As used herein, standardless analysis refers to a determination based upon an internal standard. For example, the frequency of a polymorphism can be determined herein by comparing signals within a single mass spectrum.

[0110] As used herein, amplifying refers to methods for increasing the amount of a bipolymer, especially nucleic acids. Based on the 5' and 3' primers that are chosen, amplication also serves to restrict and define the region of the genome which is subject to analysis. Amplification can be performed by any method known to those skilled in the art, including use of the polymerase chain reaction (PCR) etc. Amplification, e.g., PCR must be done quantitatively when the frequency of polymorphism is required to be determined.

[0111] As used herein, cleaving refers to non-specific and specific fragmentation of a biopolymer.

[0112] As used herein, multiplexing refers to the simultaneous detection of more than one polymorphism. Methods for performing multiplexed reactions, particularly in conjunction with mass spectrometry are known (see, e.g., U.S. Pat. Nos. 6,043,031, 5,547,835 and International PCT application No. WO 97/37041).

[0113] As used herein, reference to mass spectrometry encompasss any suitable mass spectrometric format known to those of skill in the art. Such formats inlude, but are not limited to, Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI (see, e.g., published International PCT application No.99/57318 and U.S. Pat. No. 5,118,937), Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof. MALDI, particular UV and IR, are among the formats contemplated.

[0114] As used herein, mass spectrum refers to the presentation of data obtained from analyzing a biopolymer or fragment thereof by mass spectrometry either graphically or encoded numerically.

[0115] As used herein, a blood component is a component that is separated from blood and includes, but is not limited to red blood cells and platelets, blood clotting factors, plasma, enzymes, plasminogen, immunoglobulins. A cellular blood component is a component of blood, such as a red blood cell, that is a cell. A blood protein is a protein that is normally found in blood. Examples of such proteins are blood factors VII and VIII. Such proteins and components are well-known to those of skill in the art.

[0116] As used herein, plasma can be prepared by any method known to those of skill in the art. For example, it can be prepared by centrifuging blood at a force that pellets the red cells and forms an interface between the red cells and the buffy coat, which contains leukocytes, above which is the plasma. For example, typical platelet concentrates contain at least about 10% plasma.

[0117] Blood can be separated into its components, including, but not limited to, plasma, platelets and red blood cells by any method known to those of skill in the art. For example, blood can be centrifuged for a sufficient time and at a sufficient acceleration to form a pellet containing the red blood cells. Leukocytes collect primarily at the interface of the pellet and supernatant in the buffy coat region. The supernatant, which contains plasma, platelets, and other blood components, can then be removed and centrifuged at a higher acceleration, whereby the platelets pellet.

[0118] As used herein, p53 is a cell cycle control protein that assesses DNA damage and acts as a transcription factor regulation gene which control cell growth, DNA repair and apoptosis. The p53 mutations have been found in a wide variety of different cancers, including all of the different types of leukemia, with varying frequency. The loss of normal p53 functions results in genomic instability and uncontrolled growth of the host cell.

[0119] As used herein, p21 is a cyclin-dependent kinase inhibitor, associated with G1 phase arrest of normal cells. Expression triggers apoptosis or programmed cell death and has been associated with Wilms' tumor, a pediatric kidney cancer.

[0120] As used herein, Factor VII is a serine protease involved the extrinsic blood coagulation cascade. This factor is activated by thrombin and works with tissue factor (Factor III) in the processing of Factor X to Factor Xa. Evidence has supported an association between polymorphisms in the gene and increase Factor VII activity which can result in an elevated risk of ischemic cardiovascular disease including myocardial infarction.

[0121] As used herein, a relational database stores information in a form representative of matrices, such as two-dimensional tables, including rows and columns of data, or higher dimensional matrices. For example, in one embodiment, the relational database has separate tables each with a parameter. The tables are linked with a record number, which also acts as an index. The database can be searched or sorted by using data in the tables and is stored in any suitable storage medium, such as floppy disk, CD rom disk, hard drive or other suitable medium.

[0122] As used herein, a bar codes refers any array of optically readable marks of any desired size and shape that are arranged in a reference context or frame of, typically, although not necessarily, one or more columns and one or more rows. For purposes herein, the bar code refers to any symbology, not necessary "bar" but can include dots, characters or any symbol or symbols.

[0123] As used herein, symbology refers to an identifier code or symbol, such as a bar code, that is linked to a sample. The index will reference each such symbology. The symbology is any code known or designed by the user. The symbols are associated with information stored in the database. For example, each sample can be uniquely identified with an encoded symbology. The parameters, such as the answers to the questions and subsequent genotypic and other information obtained upon analysis of the samples is included in the database and associated with the symbology. The database is stored on any suitable recording medium, such as a hard drive, a floppy disk, a tape, a CD ROM, a DVD disk and any other suitable medium.

[0124] DATABASES

[0125] Human genotyping is currently dependent on collaborations with hospitals, tissues banks and research institutions that provide samples of disease tissue. This approach is based on the concept that the onset and/or progression of diseases can be correlated with the presence of a polymorphisms or other genetic markers. This approach does not consider that disease correlated with the presence of specific markers and the absence of specific markers. It is shown herein that identification and scoring of the appearance and disappearance of markers is possible only if these markers are measured in the background of healthy subjects where the onset of disease does not mask the change in polymorphism occurrence. Databases of information from disease populations suffer from small sample size, selection bias and heterogeneity. The databases provided herein from healthy populations solve these problems by permitting large sample bands, simple selection methods and diluted heterogeneity.

[0126] Provided herein are first databases of parameters, associated with non-selected, particularly healthy, subjects. Also provided are combinations of the databases with indexed samples obtained from each of the subjects. Further provided are databases produced from the first databases.

These contain, in addition to the original parameters, information, such as genotypic information, including, but are not limited to, genomic sequence information, derived from the samples.

[0127] The databases, which are herein designated healthy databases, are so-designated because they are not obtained from subjects pre-selected for a particular disease. Hence, although individual members can have a disease, the collection of individuals is not selected to have a particular disease.

[0128] The subjects from whom the parameters are obtained comprise either a set of subjects who are randomly selected across, typically, all populations, or are pre-selected to be disease-free or healthy. As a result, the database is not selected to be representative of any pre-selected phenotype, genotype, disease or other characteristic. Typically the number of subjects from which the database is prepared is selected to produce statistically significant results when used in the methods provided herein. Generally, the number of subjects will be greater than 100, 200, and typically than 1000. The precise number can be empirically determined based upon the frequency of the parameter(s) that can be used to sort the database. Generally the population can have at least 50, at least 100, at least 200, at least 500, at least 1000, at least 5000 or at least 10,000 or more subjects.

[0129] Upon identification of a collection of subjects, information about each subject is recorded and associated with each subject as a database. The information associated with each of the subjects, includes, but is not limited to, information related to historical characteristics of the subjects, phenotypic characteristics and also genotypic characteristics, medical characteristics and any other traits and characteristics about the subject that can be determined. This information will serve as the basis for sorting the database.

[0130] In an exemplary embodiment, the subjects are mammals, such as humans, and the information relates to one or more of parameters, such as age, sex, medical history, ethnicity and any other factor. Such information, when the animals are humans, for example, can be obtained by a questionnaire and by observations about the individual, such as hair color, eye color and other characteristics. Genotypic information can be obtained from tissue or other body and body fluid samples from the subject.

[0131] The healthy genomic database can include profiles and polymorphisms from healthy individuals from a library of blood samples where each sample in the library is an individual and separate blood or other tissue sample. Each sample in the database is profiled as to the sex, age, ethnic group, and disease history of the donor.

[0132] The databases are generated by first identifying healthy populations of subjects and obtaining information about each subject that will serve as the sorting parameters for the database. This information can be entered into a storage medium, such as the memory of a computer.

[0133] The information obtained about each subject in a population used for generating the database is stored in a computer memory or other suitable storage medium. The information is linked to an identifier associated with each subject. Hence the database will identify a subject, for example by a datapoint representative of a bar code, and then all information, such as the information from a questionnaire, regarding the individual is associated with the datapoint. As the information is collected the database is generated.

[0134] Thus, for example, profile information, such as subject histories obtained from questionnaires, is collected in the database. The resulting database can be sorted as desired, using standard software, such as by age, sex and/or ethnicity. An exemplary questionnaire for subjects from whom samples are to be obtained is shown in FIGS. **22A-D**. Each questionnaire, for example, can be identified by a bar code, particularly a machine readable bar code for entry into the database. After a subject provides data and is deemed to be healthy (i.e., meets standards for blood donation), the data in the questionnaire is entered into the database and is associated with the bar code. A tissue, cell or blood sample is obtained from the subject.

[0135] **FIG. 4** exemplifies processing and tracking of blood sample components. Each component is tracked with a bar code, dated, is entered into the database and associated with the subject and the profile of the subject. Typically, the whole blood is centrifuged to produce plasma, red blood cells (which pellet) and leukocytes found in the buffy coat which layers in between. Various samples are obtained and coded with a bar code and stored for use as needed.

[0136] Samples are collected from the subjects. The samples include, but are not limited to, tissues, cells, and fluids, such as nucleic acid, blood, plasma, amniotic fluid, synovial fluid, urine, saliva, aqueous humor, sweat, sperm samples and cerebral spinal fluid. It is understood that the particular set of samples depends upon the organisms in the population.

[0137] Once samples are obtained the collection can be stored and, in some embodiments, each sample is indexed with an identifier, particularly a machine readable code, such as a bar code. For analyses, the samples or components of the samples, particularly biopolymers and small molecules, such as nucleic acids and/or proteins and metabolites, are isolated.

[0138] After samples are analyzed, this information is entered into the database in the memory of the storage medium and associated with each subject. This information includes, but is not limited to, genotypic information. Particularly, nucleic acid sequence information and other information indicative of polymorphisms, such as masses of PCR fragments, peptide fragment sequences or masses, spectra of biopolymers and small molecules and other indicia of the structure or function of a gene, gene product or other marker from which the existence of a polymorphism within the population can be inferred.

[0139] In an exemplary embodiment, a database can be derived from a collection of blood samples. For example, **FIG. 1** (see, also **FIG. 10**) shows the status of a collection of over 5000 individual samples. The samples were processed in the laboratory following SOP (standard operating procedure) guidelines. Any standard blood processing protocol can be used.

[0140] For the exemplary database described herein, the following criteria were used to select subjects:

[0141] No testing is done for infectious agents.

[0142] Age: At least 17 years old

[0143] Weight: Minimum of 110 pounds

[0144] Permanently Disqualified:

[0145] History of hepatitis (after age 11)

[0146] Leukemia Lymphoma

[0147] Human immunodeficiency virus (HIV),AIDS

[0148] Chronic kidney disease

[0149] Temporarily Disqualified:

[0150] Pregnancy—until six weeks after delivery, miscarriage or abortion

[0151] Major surgery or transfusions—for one year

[0152] Mononucleosis—until complete recovery

[0153] Prior whole blood donation—for eight weeks

[0154] Antibiotics by injection for one week; by mouth, for forty-eight hours, except antibiotics for skin complexion;

[0155] 5 year Deferment:

[0156] Internal cancer and skin cancer if it has been removed, is healed and there is no recurrence

[0157] These correspond to blood bank criteria for donating blood and represent a healthy population as defined herein for a human healthy database.

[0158] Structure of the Database

[0159] Any suitable database structure and format known to those of skill in the art can be employed. For example, a relational database is a an exemplary format in which data are stored as matrices or tables of the parameters linked by an indexer that identifies each subject. Software for preparing and manipulating, including sorting the database, can be readily developed or adapted from commercially available software, such as Microsoft Access.

[0160] Quality Control

[0161] Quality control procedures can be implemented. For example, after collection of samples, the quality of the collection in the bank can be assessed. For example, mix-up of samples can be checked by testing for known markers, such as sex. After samples are separated by ethnicity, samples are randomly tested for a marker associated with a particular ethnicity, such as HLA DQA1 group specific component, to assess whether the samples have been properly sorted by ethnic group. An exemplary sample bank is depicted in **FIG. 4.**

[0162] Obtaining Genotypic Data and Other Parameters for the Database

[0163] After informational and historical parameters are entered into the database, material from samples obtained from each subject, is analyzed. Analyzed material include proteins, metabolites, nucleic acids, lipids and any other desired constituent of the material. For example, nucleic acids, such as genomic DNA, can be analyzed by sequencing.

[0164] Sequencing can be performed using any method known to those of skill in the art. For example, if a polymorphism is identified or known, and it is desired to assess its frequency or presence among the subjects in the database, the region of interest from each sample can be isolated, such as by PCR or restriction fragments, hybridization or other suitable method known to those of skill in the art and sequenced. For purposes herein, sequencing analysis can be effected using mass spectrometry (see, e.g., U.S. Pat. Nos. 5,547,835, 5,622,824, 5,851,765, and 5,928,906). Nucleic acids also can be sequenced by hybridization (see, e.g., U.S. Pat. Nos. 5,503,980, 5,631,134, 5,795,714) and including analysis by mass spectrometry (see, U.S. application Ser. Nos. 08/419,994 and 09/395,409).

[0165] In other detection methods, it is necessary to first amplify prior to identifying the allelic variant. Amplification can be performed, e.g., by PCR and/or LCR, according to methods known in the art. In one embodiment, genomic DNA of a cell is exposed to two PCR primers and amplification for a number of cycles sufficient to produce the required amount of amplified DNA. In some embodiments, the primers are located between 150 and 350 base pairs apart.

[0166] Alternative amplification methods include: self sustained sequence replication (Guatelli, J. C. et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:1173-1177), Q-Beta Replicase (Lizardi, P. M. et al., 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

[0167] Nucleic acids also can be analyzed by detection methods and protocols, particularly those that rely on mass spectrometry (see, e.g., U.S. Pat. No. 5,605,798, 6,043,031, allowed copending U.S. application Ser. No. 08/744,481, U.S. application Ser. No. 08/990,851 and International PCT application No. WO 99/31278, International PCT application No. WO 98/2001 9). These methods can be automated (see, e.g., copending U.S. application Ser. No. 09/285,481 and published International PCT application No. PCT/US00/08111, which describes an automated process line). Among the methods of analysis herein are those involving the primer oligo base extension (PROBE) reaction with mass spectrometry for detection (described herein and elsewhere, see e.g., U.S. Pat. No. 6,043,031; see, also U.S. application Ser. Nos. 09/287,681, 09/287,682, 09/287,141 and 09/287,679, allowed copending U.S. application Ser. No. 08/744,481, International PCT application No. PCT/US97/20444, published as International PCT application No. WO 98/20019, and based upon U.S. application Ser. Nos. 08/744,481, 08/744,590, 08/746,036, 08/746,055, 08/786,988, 08/787,639, 08/933,792, 08/746,055, 08/786,988 and 08/787,639; see, also U.S. application Ser. No. 09/074,936, U.S. Pat. No. 6,024,925, and U.S. application Ser. Nos. 08/746,055 and 08/786,988, and published International PCT application No. WO 98/20020)

[0168] A chip based format in which the biopolymer is linked to a solid support, such as a silicon or silicon-coated substrate, such as in the form of an array, is among the formats for performing the analyses is. Generally, when analyses are performed using mass spectrometry, particularly MALDI, small nanoliter volumes of sample are loaded on, such that the resulting spot is about, or smaller than, the

size of the laser spot. It has been found that when this is achieved, the results from the mass spectrometric analysis are quantitative. The area under the signals in the resulting mass spectra are proportional to concentration (when normalized and corrected for background). Methods for preparing and using such chips are described in U.S. Pat. No. 6,024,925, co-pending U.S. application Ser. Nos. 08/786, 988, 09/364,774, 09/371,150 and 09/297,575; see, also U.S. application Ser. No. PCT/US97/20195, which published as WO 98/20020. Chips and kits for performing these analyses are commercially available from SEQUENOM under the trademark MassARRAY. MassArray relies on the fidelity of the enzymatic primer extension reactions combined with the miniaturized array and MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry to deliver results rapidly. It accurately distinguishes single base changes in the size of DNA fragments associated with genetic variants without tags.

[0169] The methods provided herein permit quantitative determination of alleles. The areas under the signals in the mass spectra can be used for quantitative determinations. The frequency is determined from the ratio of the signal to the total area of all of the spectrum and corrected for background. This is possible because of the PROBE technology as described in the above applications incorporated by reference herein.

[0170] Additional methods of analyzing nucleic acids include amplification-based methods including polymerase chain reaction (PCR), ligase chain reaction (LCR), mini-PCR, rolling circle amplification, autocatalytic methods, such as those using Qβ replicase, TAS, 3SR, and any other suitable method known to those of skill in the art.

[0171] Other methods for analysis and identification and detection of polymorphisms, include but are not limited to, allele specific probes, Southern analyses, and other such analyses.

[0172] The methods described below provide ways to fragment given amplified or non-amplified nucleotide sequences thereby producing a set of mass signals when mass spectrometry is used to analyze the fragment mixtures.

[0173] Amplified fragments are yielded by standard polymerase chain methods (U.S. Pat. Nos. 4,683,195 and 4,683, 202). The fragmentation method involves the use of enzymes that cleave single or double strands of DNA and enzymes that ligate DNA. The cleavage enzymes can be glycosylases, nickases, and site-specific and non site-specific nucleases, such as, but are not limited to, glycosylases, nickases and site-specific nucleases.

[0174] Glycosylase Fragmentation Method

[0175] DNA glycosylases specifically remove a certain type of nucleobase from a given DNA fragment. These enzymes can thereby produce abasic sites, which can be recognized either by another cleavage enzyme, cleaving the exposed phosphate backbone specifically at the abasic site and producing a set of nucleobase specific fragments indicative of the sequence, or by chemical means, such as alkaline solutions and or heat. The use of one combination of a DNA glycosylase and its targeted nucleotide would be sufficient to generate a base specific signature pattern of any given target region.

[0176] Numerous DNA glcosylases are known, For example, a DNA glycosylase can be uracil-DNA glycolsylase (UDG), 3-methyladenine DNA glycosylase, 3-methyladenine DNA glycosylase II, pyrimidine hydrate-DNA glycosylase, FaPy-DNA glycosylase, thymine mismatch-DNA glycosylase, hypoxanthine-DNA glycosylase, 5-Hydroxymethyluracil DNA glycosylase (HmUDG), 5-Hydroxymethylcytosine DNA glycosylase, or 1,N6-etheno-adenine DNA glycosylase (see, e.g.,, U.S. Pat. Nos. 5,536,649, 5,888,795, 5,952,176 and 6,099,553, International PCT application Nos. WO 97/03210, WO 99/54501; see, also, Eftedal et al. (1993) Nucleic Acids Res 21:2095-2101, Bjelland and Seeberg (1987) Nucleic Acids Res. 15:2787-2801, Saparbaev et al. (1995) Nucleic Acids Res. 23:3750-3755, Bessho (1999) Nucleic Acids Res. 27:979-983) corresponding to the enzyme's modified nucleotide or nucleotide analog target. uracil-DNA glycolsylase (UDG) is an exemplary glycosylase.

[0177] Uracil, for example, can be incorporated into an amplified DNA molecule by amplifying the DNA in the presence of normal DNA precursor nucleotides (e.g. dCTP, dATP, and dGTP) and dUTP. When the amplified product is treated with UDG, uracil residues are cleaved. Subsequent chemical treatment of the products from the UDG reaction results in the cleavage of the phosphate backbone and the generation of nucleobase specific fragments. Moreover, the separation of the complementary strands of the amplified product prior to glycosylase treatment allows complementary patterns of fragmentation to be generated. Thus, the use of dUTP and Uracil DNA glycosylase allows the generation of T specific fragments for the complementary strands, thus providing information on the T as well as the A positions within a given sequence. Similar to this, a C-specific reaction on both (complementary) strands (i.e. with a C-specific glycosylase) yields information on C as well as G positions within a given sequence if the fragmentation patterns of both amplification strands are analyzed separately. Thus, with the glycosylase method and mass spectrometry, a full series of A, C, G and T specific fragmentation patterns can be analyzed.

[0178] Nickase Fragmentation Method

[0179] A DNA nickase, or DNase, can be used to recognize and cleave one strand of a DNA duplex. Numerous nickases are known. Among these, for example, are nickase NY2A nickase and NYS1 nickase (Megabase) with the following cleavage sites:

[0180] NY2A:

[0181] 5' . . . R AG . . . 3'

[0182] 3' . . . Y TC . . . 5' where R=A or G and Y=C or T

[0183] NYS1:

[0184] 5' . . . CC[A/G/T] . . . 3'

[0185] 3' . . . GG[T/C/A] . . . 5'.

[0186] Fen-Ligase Fragmentation Method

[0187] The Fen-ligase method involves two enzymes: Fen-1 enzyme and a ligase. The Fen-1 enzyme is a site-specific nuclease known as a "flap" endonuclease (U.S. Pat. Nos. 5,843,669, 5,874,283, and 6,090,606). This enzyme recognizes and cleaves DNA "flaps" created by the overlap

of two oligonucleotides hybridized to a target DNA strand. This cleavage is highly specific and can recognize single base pair mutations, permitting detection of a single homologue from an individual heterozygous at one SNP of interest and then genotyping that homologue at other SNPs occurring within the fragment. Fen-1 enzymes can be Fen-1 like nucleases e.g. human, murine, and Xenopus XPG enzymes and yeast RAD2 nucleases or Fen-1 endonucleases from, for example, *M. jannaschii, P. furiosus,* and *P. woesei.* Among such enzymes are the Fen-1 enzymes.

[0188] The ligase enzyme forms a phosphodiester bond between two double stranded nucleic acid fragments. The ligase can be DNA Ligase I or DNA Ligase III (see, e.g., U.S. Pat. Nos. U.S. 5,506,137, 5,700,672, 5,858,705 and 5,976,806; see, also, Waga, et al. (1994) J. Biol. Chem. 269:10923-10934, Li et al. (1994) Nucleic Acids Res. 22:632-638, Arrand et al. (1986) J. Biol. Chem. 261:9079-9082, Lehman (1974) Science 186:790-797, Higgins and Cozzarelli (1979) Methods Enzymol. 68:50-71, Lasko et al. (1990) Mutation Res. 236:277-287, and Lindahl and Barnes (1992) Ann. Rev. Biochem. 61:251-281). Thermostable ligase (Epicenter Technologies), where "thermostable" denotes that the ligase retains activity even after exposure to temperatures necessary to separate two strands of DNA, are among the ligases for use herein.

[0189] Type IIS Enzyme Fragmentation Method

[0190] Restriction enzymes bind specifically to and cleave double-stranded DNA at specific sites within or adjacent to a particular recognition sequence. These enzymes have been classified into three groups (e.g. Types I, II, and III) as known to those of skill in the art. Because of the properties of type I and type III enzymes, they have not been widely used in molecular biological applications. Thus, for purposes herein type II enzymes are among those contemplated. Of the thousands of restriction enzymes known in the art, there are 179 different type II specificities. Of the 179 unique type II restriction endonucleases, 31 have a 4-base recognition sequence, 11 have a 5-base recognition sequence, 127 have a 6-base recognition sequence, and 10 have recognition sequences of greater than six bases (U.S. Pat. No. 5,604,098). Of category type II enzymes, type IIS is exemplified herein.

[0191] Type IIS enzymes can be Alw XI, Bbv I, Bce 83, Bpm I, Bsg I, Bsm AI, Bsm FI, Bsa I, Bcc I, Bcg I, Ear I, Eco 57I, Esp 3I, Fau I, Fok I, Gsu I, Hga I, Mme I, Mbo II, Sap I, and the otheres.

[0192] The Fok I enzyme endonuclease is an exemplary well characterized member of the Type IIS class (see, e.g., U.S. Pat. Nos. 5,714,330, 5,604,098, 5,436,150, 6,054,276 and 5,871,911; see, also, Szybalski et al. (1991) Gene 100:13-26, Wilson and Murray (1991) Ann. Rev. Genet. 25:585-627, Sugisaki et al. (1981) Gene 16:73-78, Podhajska and Szalski (1985) Gene 40:175-182. Fok I recognizes the sequence 5'GGATG-3' and cleaves DNA accordingly. Type IIS restriction sites can be introduced into DNA targets by incorporating the sites into primers used to amplify such targets. Fragments produced by digestion with Fok I are site specific and can be analyzed by mass spectrometry methods such as MALDI-TOF mass spectrometry, ESI-TOF mass spectrometry, and any other type of mass spectrometry well known to those of skill in the art.

[0193] Once a polymorphism has been found to correlate with a parameter such as age, age groups can be screened for polymorphisms. The possibility of false results due to allelic dropout is examined by doing comparative PCR in an adjacent region of the genome.

[0194] Analyses

[0195] In using the database, allelic frequencies can be determined across the population by analyzing each sample in the population individually, determining the presence or absence of allele or marker of interest in each individual sample, and then determining the frequency of the marker in the population. The database can then be sorted (stratified) to identify any correlations between the allele and a selected parameter using standard statistical analysis. If a correlation is observed, such as a decrease in a particular marker with age or correlation with sex or other parameter, then the marker is a candidate for further study, such as genetic mapping to identify a gene or pathway in which it is involved. The marker can then be correlated, for example, with a disease. Haplotying also can be carried out. Genetic mapping can be effected using standard methods and can also require use of databases of others, such as databases previously determined to be associated with a disorder.

[0196] Exemplary analyses have been performed and these are shown in the figures, and discussed herein.

[0197] Sample Pooling

[0198] It has been found that using the databases provided herein, or any other database of such information, substantially the same frequencies that were obtained by examining each sample separately can be obtained by pooling samples, such as in batches of 10, 20, 50, 100, 200, 500, 1000 or any other number. A precise number can be determined empirically if necessary, and can be as low as 3.

[0199] In one embodiment, the frequency of genotypic and other markers can be obtained by pooling samples. To do this a target population and a genetic variation to be assessed is selected, a plurality of samples of biopolymers are obtained from members of the population, and the biopolymer from which the marker or genotype can be inferred is determined or detected. A comparison of samples tested in pools and individually and the sorted results therefrom are shown in **FIG. 9**, which shows frequency of the factor VII Allele 353Q. **FIG. 10** depicts the frequency of the CETP Allele in pooled versus individual samples. **FIG. 15** shows ethnic diversity among various ethnic groups in the database using pooled DNA samples to obtain the data. FIGS. **12-14** show mass spectra for these samples.

[0200] Pooling of test samples has application not only to the healthy databases provided herein, but also to use in gathering data for entry into any database of subjects and genotypic information, including typical databases derived from diseased populations. What is demonstrated herein, is the finding that the results achieved are statistically the same as the results that would be achieved if each sample is analyzed separately. Analysis of pooled samples by a method, such as the mass spectrometric methods provided herein, permits resolution of such data and quantitation of the results.

[0201] For factor VII the R53Q acid polymorphism was assessed. In **FIG. 9**, the "individual" data represent allelic frequency observed in 92 individuals reactions. The pooled data represent the allelic frequency of the same 92 individu-

als pooled into a single probe reaction. The concentration of DNA in the samples of individual donors is 250 nanograms. The total concentration of DNA in the pooled samples is also 250 nanograms, where the concentration of any individual DNA is 2.7 nanograms.

[0202] It also was shown that it is possible to reduce the DNA concentration of individuals in a pooled samples from 2.7 nanograms to 0.27 nanograms without any change in the quality of the spectrum or the ability to quantitate the amount of sample detected. Hence low concentrations of sample can be used in the pooling methods.

[0203] Use of the Databases and Markers Identified Thereby

[0204] The successful use of genomics requires a scientific hypothesis (i.e., common genetic variation, such as a SNP), a study design (i.e., complex disorders), samples and technology, such as the chip-based mass spectrometric analyses (see, e.g., U.S. Pat. No. 5,605,798, U.S. Pat. No. 5,777,324, U.S. Pat. No. 6,043,031, allowed copending U.S. application Ser. No. 08/744,481, U.S. application Ser. No. 08/990,851, International PCT application No. WO 98/20019, copending U.S. application Ser. No. 09/285,481, which describes an automated process line for analyses; see, also, U.S. application Ser. Nos. 08/617,256, 09/287,681, 09/287,682, 09/287,141 and 09/287,679, allowed copending U.S. application Ser. No. 08/744,481, International PCT application No. PCT/US97/20444, published as International PCT application No. WO 98/20019, and based upon U.S. application Ser. Nos. 08/744,481, 08/744,590, 08/746,036, 08/746,055, 08/786,988, 08/787,639, 08/933,792, 08/746, 055, 09/266,409, 08/786,988 and 08/787,639; see, also U.S. application Ser. No. 09/074,936). All of these aspects can be used in conjunction with the databases provided herein and samples in the collection.

[0205] The databases and markers identified thereby can be used, for example, for identification of previously unidentified or unknown genetic markers and to identify new uses for known markers. As markers are identified, these can be entered into the database to use as sorting parameters from which additional correlations can be determined.

[0206] Previously Unidentified or Unknown Genetic Markers

[0207] The samples in the healthy databases can be used to identify new polymorphisms and genetic markers, using any mapping, sequencing, amplification and other methodologies, and in looking for polymorphisms among the population in the database. The thus-identified polymorphism can then be entered into the database for each sample, and the database sorted (stratified) using that polymorphism as a sorting parameter to identify any patterns and correlations that emerge, such as age correlated changes in the frequency of the identified marker. If a correlation is identified, the locus of the marker can be mapped and its function or effect assessed or deduced.

[0208] Thus, the databases here provide means for:

- **[0209]** identification of significantly different allelic frequencies of genetic factors by comparing the occurrence or disappearance of the markers with increasing age in population and then associating the markers with a disease or a biochemical pathway;
- **[0210]** identification of significantly different allelic frequencies of disease causing genetic factors by comparing the male with the female population or comparing other selected stratified populations and associating the markers with a disease or a biochemical pathway;
- **[0211]** identification of significantly different allelic frequencies of disease causing genetic factors by comparing different ethnic groups and associating the markers with a disease or a biochemical pathway that is known to occur in high frequency in the ethnic group;
- **[0212]** profiling potentially functional variants of genes through the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating the contribution of the variant genes to the physical condition of the investigated population;
- **[0213]** identification of functionally relevant gene variants by gene disequilibrium analysis performed within the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating their contribution to the physical condition of investigated population;
- **[0214]** identification of potentially functional variants of chromosomes or parts of chromosomes by linkage disequilibrium analysis performed within the general panmixed population stratified according to age, sex, and ethnic origin and thereby demonstrating their contribution to the physical condition of investigated population.

[0215] Uses of the Identified Markers and Known Markers

[0216] The databases can also be used in conjunction with known markers and sorted to identify any correlations. For example, the databases can be used for:

- **[0217]** determination and evaluation of the penetrance of medically relevant polymorphic markers;
- **[0218]** determination and evaluation of the diagnostic specificity of medically relevant genetic factors;
- **[0219]** determination and evaluation of the positive predictive value of medically relevant genetic factors;
- **[0220]** determination and evaluation of the onset of complex diseases, such as, but are not limited to, diabetes, hypertension, autoimmune diseases, arteriosclerosis, cancer and other diseases within the general population with respect to their causative genetic factors;
- **[0221]** delineation of the appropriate strategies for preventive disease treatment;
- **[0222]** delineation of appropriate timelines for primary disease intervention;
- **[0223]** validation of medically relevant genetic factors identified in isolated populations regarding their general applicability;
- **[0224]** validation of disease pathways including all potential target structures identified in isolated populations regarding their general applicability; and

[0225] validation of appropriate drug targets identified in isolated populations regarding their general applicability.

[0226] Among the diseases and disorders for which polymorphisms can be linked include, those linked to inborn errors of metabolism, acquired metabolic disorders, intermediary metabolism, oncogenesis pathways, blood clotting pathways, and DNA synthetic and repair pathways, DNA repair/replication/transcription factors and activities, e.g., such as genes related to oncogenesis, aging and genes involved in blood clotting and the related biochemical pathways that are related to thrombosis, embolism, stroke, myocardial infarction, angiogenesis and oncogenesis.

[0227] For example, a number of diseases are caused by or involve deficient or defective enzymes in intermediary metabolism (see, e.g., Tables 1 and 2, below) that result, upon ingestion of the enzyme substrates, in accumulation of harmful metabolites that damage organs and tissues, particularly an infant's developing brain and other organs, resulting in mental retardation and other developmental disorders.

[0228] Identification of Markers and Genes for such Disorders is of Great Interest.

[0229] Model Systems

[0230] Several gene systems, p21, p53 and Lipoprotein Lipase polymorphism (N291S), were selected. The p53 gene is a tumor suppressor gene that is mutated in diverse tumor types. One common allelic variant occurs at codon 72. A polymorphism that has been identified in the p53 gene, i.e., the R72P allele, results in an amino acid exchange, arginine to proline, at codon 72 of the gene.

[0231] Using diseased populations, it has been shown that there are ethnic differences in the allelic distribution of these alleles among African-Americans and Caucasians in the U.S. The results here support this finding and also demonstrate that the results obtained with a healthy database are meaningful (see, **FIG. 7B**).

[0232] The 291S allele leads to reduced levels of high density lipoprotein cholesterol (HDL-C) that is associated with an increased risk of males for arteriosclerosis and in particular myocardial infarction (see, Reymer et al. (1995) *Nature Genetics* 10:28-34).

[0233] Both genetic polymorphisms were profiled within a part of the Caucasian population-based sample bank. For the polymorphism located in the lipoprotein lipase gene a total of 1025 unselected individuals (436 males and 589 females) were tested. Genomic DNA was isolated from blood samples obtained from the individuals.

[0234] As shown in the Examples and figures, an exemplary database containing about 5000 subjects, answers to the questionnaire (see **FIG. 3**), and genotypic information has been stratified. A particular known allele has been selected, and the samples tested for the marker using mass spectrometric analyses, particularly PROBE (see the EXAMPLES) to identify polymorphisms in each sample. The population in the database has been sorted according to various parameters and correlations have been observed. For example, FIGS. 2A-C, show sorting of the data by age and sex for the Lipoprotein Lipase gene in the Caucasian population in the database. The results show a decrease in the frequency of the allele with age in males but no such decrease in females. Other alleles that have been tested against the database, include, alleles of p53, p21 and factor VII. Results when sorted by age are shown in the figures.

[0235] These examples demonstrate an effect of altered frequency of disease causing genetic factors within the general population. The scientific interpretation of those results allows prediction of medical relevance of polymorphic genetic alterations. In addition, conclusions can be drawn with regard to their penetrance, diagnostic specificity, positive predictive value, onset of disease, most appropriate onset of preventive strategies, and the general applicability of genetic alterations identified in isolated populations to panmixed populations.

[0236] Therefore, an age- and sex-stratified population-based sample bank that is ethnically homogenous is a suitable tool for rapid identification and validation of genetic factors regarding their potential medical utility.

[0237] Exemplary Computer System for Creating, Storing and Processing the Databases

[0238] Systems

[0239] Systems, including computers, containing the databases are provided herein. The computers and databases can be used in conjunction, for example, with the APL system (see, copending U.S. application Ser. No. 09/285,481), which is an automated system for analyzing biopolymers, particularly nucleic acids. Results from the APL system can be entered into the database.

[0240] Any suitable computer system can be used. The computer system can be integrated into systems for sample analysis, such as the automated process line described herein (see, e.g., copending U.S. application Ser. No. 09/285,481).

[0241] **FIG. 17** is a block diagram of a computer constructed to provide and process the databases described herein. The processing that maintains the database and performs the methods and procedures can be performed on multiple computers all having a similar construction, or can be performed by a single, integrated computer. For example, the computer through which data are added to the database can be separate from the computer through which the database is sorted, or can be integrated with it. In either arrangement, the computers performing the processing can have a construction as illustrated in **FIG. 17**.

[0242] **FIG. 17** is a block diagram of an exemplary computer **1700** that maintains the database described above and performs the methods and procedures. Each computer **1700** operates under control of a central processor unit (CPU) **1702**, such as a "Pentium" microprocessor and associated integrated circuit chips, available from Intel Corporation of Santa Clara, Calif., USA. A computer user can input commands and data from a keyboard and display mouse **1704** and can view inputs and computer output at a display **1706**. The display is typically a video monitor or flat panel display device. The computer **1700** also includes a direct access storage device (DASD) **1707**, such as a fixed hard disk drive. The memory **1708** typically comprises volatile semiconductor random access memory (RAM). Each computer can include a program product reader **1710** that accepts a program product storage device **1712**, from which the program product reader can read data (and to

which it can optionally write data). The program product reader can comprise, for example, a disk drive, and the program product storage device can comprise removable storage media such as a magnetic floppy disk, an optical CD-ROM disc, a CD-R disc, a CD-RW disc, or a DVD data disc. If desired, the computers can be connected so they can communicate with each other, and with other connected computers, over a network **1713**. Each computer **1700** can communicate with the other connected computers over the network **1713** through a network interface **1714** that enables communication over a connection **1716** between the network and the computer.

[0243] The computer **1700** operates under control of programming steps that are temporarily stored in the memory **1708** in accordance with conventional computer construction. When the programming steps are executed by the CPU **1702**, the pertinent system components perform their respective functions. Thus, the programming steps implement the functionality of the system as described above. The programming steps can be received from the DASD **1707**, through the program product reader **1712**, or through the network connection **1716**. The storage drive **1710** can receive a program product, read programming steps recorded thereon and transfer the programming steps into the memory **1708** for execution by the CPU **1702**. As noted above, the program product storage device **1710** can comprise any one of multiple removable media having recorded computer-readable instructions, including magnetic floppy disks and CD-ROM storage discs. Other suitable program product storage devices can include magnetic tape and semiconductor memory chips. In this way, the processing steps necessary for operation can be embodied on a program product.

[0244] Alternatively, the program steps can be received into the operating memory **1708** over the network **1713**. In the network method, the computer receives data including program steps into the memory **1708** through the network interface **1714** after network communication has been established over the network connection **1716** by well-known methods that will be understood by those skilled in the art without further explanation. The program steps are then executed by the CPU **1702** to implement the processing of the Garment Database system.

[0245] It should be understood that all of the computers of the system and can have a construction similar to that shown in **FIG. 17.** Details described with respect to the **FIG. 17** computer **1700** will be understood to apply to all computers of the system **1700**. This is indicated by multiple computers **1700** shown connected to the network **1713**. Any one of the computers **1700** can have an alternative construction, so long as they can communicate with the other computers and support the functionality described herein.

[0246] **FIG. 18** is a flow diagram that illustrates the processing steps performed using the computer illustrated in **FIG. 17**, to maintain and provide access to the databases, such as for identifying polymorphic genetic markers. In particular, the information contained in the database is stored in computers having a construction similar to that illustrated in **FIG. 17**. The first step for maintaining the database, as indicated in **FIG. 18**, is to identify healthy members of a population. As noted above, the population members are subjects that are selected only on the basis of being healthy, and where the subjects are mammals, such as humans, they can be selected based upon apparent health and the absence of detectable infections. The step of identifying is represented by the flow diagram box numbered **1802**.

[0247] The next step, represented by the flow diagram box numbered **1804**, is to obtain identifying and historical information and data relating to the identified members of the population. The information and data comprise parameters for each of the population members, such as member age, ethnicity, sex, medical history, and ultimately genotypic information. Initially, the parameter information is obtained from a questionnaire answered by each member, from whom a body tissue or body fluid sample also is obtained. The step of entering and storing these parameters into the database of the computer is represented by the flow diagram box numbered **1806**. As additional information about each population member and corresponding sample is obtained, this information can be inputted into the database and can serve as a sorting parameter.

[0248] In the next step, represented by the flow diagram box numbered **1808**, the parameters of the members are associated with an indexer. This step can be executed as part of the database storage operation, such as when a new data record is stored according to the relational database structure and is automatically linked with other records according to that structure. The step **1806** also can be executed as part of a conventional data sorting or retrieval process, in which the database entries are searched according to an input search or indexing key value to determine attributes of the data. For example, such search and sort techniques can be used to follow the occurrence of known genetic markers and then determine if there is a correlation with diseases for which they have been implicated. Examples of this use are for assessing the frequencies of the p53 and Lipoprotein Lipase polymorphisms.

[0249] Such searching of the database also can be valuable for identifying one or more genetic markers whose frequency changes within the population as a function of age, ethnic group, sex, or some other criteria. This can allow the identification of previously unknown polymorphisms and, ultimately, identification of a gene or pathway involved in the onset and progression of disease.

[0250] In addition, the database can be used for taking an identified polymorphism and ascertaining whether it changes in frequency when the data are sorted according to a selected parameter.

[0251] In this way, the databases and methods provided herein permit, among other things, identification of components, particularly key components, of a disease process by understanding its genetic underpinnings, and also an understanding of processes, such as individual drug responses. The databases and methods provided herein also can be used in methods involving elucidation of pathological pathways, in developing new diagnostic assays, identifying new potential drug targets, and in identifying new drug candidates.

[0252] Morbidity and/or Early Mortality Associated Polymorphisms

[0253] A database containing information provided by a population of healthy blood donors who were not selected for any particular disease to can be used to identify poly-

morphisms and the alleles in which they are present, whose frequency decreases with age. These can represent morbidity susceptibility markers and genes.

[0254] Polymorphisms of the genome can lead to altered gene function, protein function or genome instability. To identify those polymorphisms which have a clinical relevance/utility is the goal of a world-wide scientific effort. It can be expected that the discovery of such polymorphisms will have a fundamental impact on the identification and development of novel drug compounds to cure diseases. The strategy to identify valuable polymorphisms is cumbersome and dependent upon the availability of many large patient and control cohorts to show disease association. In particular, genes that cause a general risk of the population to suffer from any disease (morbidity susceptibility genes) will escape these case/control studies entirely.

[0255] Here described is a screening strategy to identify morbidity susceptibility genes underlying a variety of different diseases. The definition of a morbidity susceptibility gene is a gene that is expressed in many different cell types or tissues (housekeeping gene) and its altered function can facilitate the expression of a clinical phenotype caused by disease-specific susceptibility genes that are involved in a pathway specific for this disorder. In other words, these morbidity susceptibility genes predispose people to develop a distinct disease according to their genetic make-up for this disease.

[0256] Candidates for morbidity susceptibility genes can be found at the bottom level of pathways involving transcription, translation, heat-shock proteins, protein trafficking, DNA repair, assembly systems for subcellular structures (e.g. mitochondria, peroxysomes and other cellular microbodies), receptor signaling cascades, immunology, etc. Those pathways control the quality of life at the cellular level as well as for the entire organism. Mutations/polymorphisms located in genes encoding proteins for those pathways can reduce the fitness of cells and make the organism more susceptible to express the clinical phenotype caused by the action of a disease-specific susceptibility gene. Therefore, these morbidity susceptibility genes can be potentially involved in a whole variety of different complex diseases if not in all. Disease-specific susceptibility genes are involved in pathways that can be considered as disease-specific pathways like glucose-, lipid, hormone metabolism, etc.

[0257] The exemplified method permit, among other things, identification of genes and/or gene products involved in a man's general susceptibility to morbidity and/or mortality; use of these genes and/or gene products in studies to elucidate the genetic underpinnings of human diseases; use of these genes and/or gene products in combinatorial statistical analyses without or together with disease-specific susceptibility genes; use of these genes and/or gene products to predict penetrance of disease susceptibility genes; use of these genes and/or gene products in predisposition and/or acute medical diagnostics and use of these genes and/or gene products to develop drugs to cure diseases and/or to extend the life span of humans.

[0258] SCREENING PROCESS

[0259] The healthy population stratified by age, gender and ethnicity, etc. is a very efficient and a universal screening tool for morbidity associated genes. Changes of allelic frequencies in the young compared to the old population are expected to indicate putative morbidity susceptibility genes. Individual samples of this healthy population base can be pooled to further increase the throughput. In an experiment, pools of young and old Caucasian females and males were applied to screen more than 400 randomly chosen single nucleotide polymorphisms located in many different genes. Candidate polymorphisms were identified if the allelic difference was greater than 8% between young and old for both or only one of the genders. The initial results were assayed again in at least one independent subsequent experiments. Repeated experiments are necessary to recognize unstable biochemical reactions, which occur with a frequency of about 2-3% and can mimic age-related allelic frequency differences. Average frequency differences and standard deviations are calculated after successful reproducibility of initial results. The final allelic frequency is then compared to a reference population of Caucasian CEPH sample pool. The result should show similar allelic frequencies in the young Caucasian population. Subsequently, the exact allele frequencies of candidates including genotype information were obtained by analyzing all individual samples. This procedure is straight forward with regard to time and cost. It enables the screening of an enormous number of SNPs. So far, several markers with a highly significant association to age were identified and described below.

[0260] In general at least 5 individuals in a stratified population should to be screened to produce statistically significant results. The frequency of the allele is determined for an age stratified population. Chi square analysis is then performed on the allelic frequencies to determine if the difference between age groups is statistically significant. A p value less than of 0.1 is considered to represent a statistically significant difference. Typically the p value should be less than 0.05.

[0261] Clinical Trials

[0262] The identification of markers whose frequency in a population decreases with age also allows for better designed and balanced clinical trials. Currently, if a clinical trial utilizes a marker as a significant endpoint in a study and the marker disappears with age, then the results of the study can be inaccurate. By using methods provided herein, it can be ascertained that if a marker decreases in frequency with age. This information can be considered and controlled when designing the study. For, example, an age independent marker could be substituted in its place.

[0263] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

[0264] This example describes the use of a database containing information provided by a population of healthy blood donors who were not selected for any particular disease to determine the distribution of allelic frequencies of known genetic markers with age and by sex in a Caucasian subpopulation of the database. The results described in this example demonstrate that a disease-related genetic marker or polymorphism can be identified by sorting a healthy database by a parameter or parameters, such as age, sex and ethnicity.

[0265] Generating a Database

[0266] Blood was obtained by venous puncture from human subjects who met blood bank criteria for donating blood. The blood samples were preserved with EDTA at pH 8.0 and labeled. Each donor provided information such as age, sex, ethnicity, medical history and family medical history. Each sample was labeled with a barcode representing identifying information. A database was generated by entering, for each donor, the subject identifier and information corresponding to that subject into the memory of a computer storage medium using commercially available software, e.g., Microsoft Access.

[0267] Model Genetic Markers

[0268] The frequencies of polymorphisms known to be associated at some level with disease were determined in a subpopulation of the subjects represented in the database. These known polymporphisms occur in the p21, p53 and Lipoprotein Lipase genes. Specifically, the N291S polymorphism (N291S) of the Lipoprotein Lipase gene, which results in a substitution of a serine for an asparagine at amino acid codon 291, leads to reduced levels of high density lipoprotein cholesterol (HDL-C) that is associated with an increased risk of males for arteriosclerosis and in particular myocardial infarction (see, Reymer et al. (1995) *Nature Genetics* 10:28-34).

[0269] The p53 gene encodes a cell cycle control protein that assesses DNA damage and acts as a transcription factor regulating genes that control cell growth, DNA repair and apoptosis (programmed cell death). Mutations in the p53 gene have been found in a wide variety of different cancers, including different types of leukemia, with varying frequency. The loss of normal p53 function results in genomic instability an uncontrolled cell growth. A polymorphism that has been identified in the p53 gene, i.e., the R72P allele, results in the substitution of a proline for an arginine at amino acid codon 72 of the gene.

[0270] The p21 gene encodes a cyclin-dependent kinase inhibitor associated with G1 phase arrest of normal cells. Expression of the p21 gene triggers apoptosis. Polymorphisms of the p21 gene have been associated with Wilms' tumor, a pediatric kidney cancer. One polymorphism of the p21 gene, the S31R polymorphism, results in a substitution of an arginine for a serine at amino acid codon 31.

[0271] Database Analysis

Sorting of Subjects According to Specific Parameters

[0272] The genetic polymorphisms were profiled within segments of the Caucasian subpopulation of the sample bank. For p53 profiling, the genomic DNA isolated from blood from a total of 1277 Caucasian subjects age 18-59 years and 457 Caucasian subjects age 60-79 years was analyzed. For p21 profiling, the genomic DNA isolated from blood from a total of 910 Caucasian subjects age 18-49 years and 824 Caucasian subjects age 50-79 years was analyzed. For lipoprotein lipase gene profiling, the genomic DNA from a total of 1464 Caucasian females and 1470 Caucasian males under 60 years of age and a total of 478 Caucasian females and 560 Caucasian males over 60 years of age was analyzed.

Isolation and Analysis of Genomic DNA

[0273] Genomic DNA was isolated from blood samples obtained from the individuals. Ten milliliters of whole blood from each individual was centrifuged at 2000×g. One milliliter of the buffy coat was added to 9 ml of 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM Na_2EDTA, incubated 10 min at room temperature and centrifuged for 10 min at 2000×g. The supernatant was removed, and the white cell pellet was washed in 155 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM Na_2EDTA and resuspended in 4.5 ml of 50 mM Tris, 5 mM EDTA and 1% SDS. Proteins were precipitated from the cell lysate by 6 mM ammonium acetate, pH 7.3, and then separated from the nucleic acids by centrifugation at 3000×g. The nucleic acid was recovered from the supernatant by the addition of an equal volume of 100% isopropanol and centrifugation at 2000×g. The dried nucleic acid pellet was hydrated in 10 mM Tris, pH 7.6, and 1 mM Na_2EDTA and stored at 4° C.

[0274] Assays of the genomic DNA to determine the presence or absence of the known genetic markers were developed using the BiomassPROBE™ detection method (primer oligo base extension) reaction. This method uses a single detection primer followed by an oligonucleotide extension step to give products, which can be readily resolved by mass spectrometry, and, in particular, MALDI-TOF mass spectrometry. The products differ in length depending on the presence or absence of a polymorphism. In this method, a detection primer anneals adjacent to the site of a variable nucleotide or sequence of nucleotides, and the primer is extended using a DNA polymerase in the presence of one or more dideoxyNTPs and, optionally, one or more deoxyNTPs. The resulting products are resolved by MALDI-TOF mass spectrometry. The mass of the products as measured by MALDI-TOF mass spectrometry makes possible the determination of the nucleotide(s) present at the variable site.

[0275] First, each of the Caucasian genomic DNA samples was subjected to nucleic acid amplification using primers corresponding to sites 5' and 3' of the polymorphic sites of the p21 (S31R allele), p53 (R72P allele) and Lipoprotein Lipase (N291S allele) genes. One primer in each primer pair was biotinylated to permit immobilization of the amplification product to a solid support. Specifically, the polymerase chain reaction primers used for amplification of the relevant segments of the p21, p53 and lipoprotein lipase genes are shown below: US4p21c31-2F (SEQ ID NO: 9) and US5p21-2R (SEQ ID NO: 10) for p21 gene amplification; US4-p53-ex4-F (also shown as p53-ex4US4 (SEQ ID NO: 2)) and US5-p53/2-4R (also shown as US5P53/4R (SEQ ID NO: 3)) for p53 gene amplification; and US4-LPL-F2 (SEQ ID NO: 16) and US5-LPL-R2 (SEQ ID NO: 17) for lipoprotein lipase gene amplification.

[0276] Amplification of the respective DNA sequences was conducted according to standard protocols. For example, primers can be used in a concentration of 8 pmol. The reaction mixture (e.g., total volume 50 μl) can contain Taq-polymerase including 10×buffer and dTNPs. Cycling conditions for polymerase chain reaction amplification can typically be initially 5 min. at 95° C., followed by 1 min. at 94° C., 45 sec at 53° C., and 30 sec at 72° C. for 40 cycles with a final extension time of 5 min at 72° C. Amplification products can be purified by using Qiagen's PCR purification

kit (No. 28106) according to manufacturer's instructions. The elution of the purified products from the column can be done in 50 μl TE-buffer (10 mM Tris, 1 mM EDTA, pH 7.5).

[0277] The purified amplification products were immobilized via a biotin-avidin linkage to streptavidin-coated beads and the double-stranded DNA was denatured. A detection primer was then annealed to the immobilized DNA using conditions such as, for example, the following: 50 μl annealing buffer (20 mM Tris, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_2$, 1% Triton X-100, pH 8) at 50° C. for 10 min, followed by washing of the beads three times with 200 μl washing buffer (40 mM Tris, 1 mM EDTA, 50 mM NaCl, 0.1% Tween 20, pH 8.8) and once in 200 μl TE buffer.

[0278] The PROBE extension reaction was performed, for example, by, using some components of the DNA sequencing kit from USB (No. 70770) and dNTPs or ddNTPs from Pharmacia. An exemplary protocol could include a total reaction volume of 45 μl, containing of 21 μl water, 6 μl Sequenase-buffer, 3 μl 10 mM DTT solution, 4.5 μl, 0.5 mM of three dNTPs, 4.5 μl, 2 mM the missing one ddNTP, 5.5 μl glycerol enzyme dilution buffer, 0.25 μl Sequenase 2.0, and 0.25 pyrophosphatase. The reaction can then by pipetted on ice and incubated for 15 min at room temperature and for 5 min at 37° C. The beads can be washed three times with 200 μl washing buffer and once with 60 μl of a 70 mM NH_4-Citrate solution.

[0279] The DNA was denatured to release the extended primers from the immobilized template. Each of the resulting extension products was separately analyzed by MALDI-TOF mass spectrometry using 3-hydroxypicolinic acid (3-HPA) as matrix and a UV laser.

[0280] Specifically, the primers used in the PROBE reactions are as shown below: P21/31-3 (SEQ ID NO: 12) for PROBE analysis of the p21 polymorphic site; P53/72 (SEQ ID NO: 4) for PROBE analysis of the p53 polymorphic site; and LPL-2 for PROBE analysis of the lipoprotein lipase gene polymorphic site. In the PROBE analysis of the p21 polymorphic site, the extension reaction was performed using dideoxy-C. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 31 encodes a serine) and from the reaction conducted on a polymorphic S31R allele template (wherein codon 31 encodes an arginine) are shown below and designated as P21/31-3 Ser (wt) (SEQ ID NO: 13) and P21/31-3 Arg (SEQ ID NO: 14), respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 4900.2 Da for the wild-type product and 5213.4 Da for the polymorphic product).

[0281] In the PROBE analysis of the p53 polymorphic site, the extension reaction was performed using dideoxy-C. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 72 encodes an arginine) and from the reaction conducted on a polymorphic R72P allele template (wherein codon 72 encodes a proline) are shown below and designated as Cod72 G Arg (wt) and Cod72 C Pro, respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 5734.8 Da for the wild-type product and 5405.6 Da for the polymorphic product).

[0282] In the PROBE analysis of the lipoprotein lipase gene polymorphic site, the extension reaction was performed using a mixture of ddA and ddT. The products resulting from the reaction conducted on a "wild-type" allele template (wherein codon 291 encodes an asparagine) and from the reaction conducted on a polymorphic N291S allele template (wherein codon 291 encodes a serine) are shown below and designated as 291Asn and 291Ser, respectively. The masses for each product as can be measured by MALDI-TOF mass spectrometry are also provided (i.e., 6438.2 Da for the wild-type product and 6758.4 Da for the polymorphic product).

```
                         P53-1 (R72P)
                  PCR Product length: 407 bp

                         US4-p53-ex4-F            (SEQ ID NO: 1)
                         ctg aggacctggt cctctgactg
ctcttttcac ccatctacagtcccccttgccgtcccaagc aat
ggatgatttgatgctgt

ccccggacga tattgaacaa tggttcactg aagacccagg tccagatgaa gctc-
ccagaa
 P53/72           72R
tgccagaggctgctccccgc gtggcccctg caccagcagc tcctacaccg gcggc-
ccctg

                 c 72P
caccagcccc ctcctggccc ctgtcatctt ctgtcccttc ccagaaaacc tac-
cagggca

gctacggttt ccgtctgggc ttcttgcatt ctgggacagc caagtctgtg acttg-
cacgg

tcagttgccc tgaggggctg gcttccatga gacttcaa
                      US5-p53/2-4R
```

[0283]

Primers (SEQ ID NOs: 2-4)	
p53-ex4FUS4	cccagtcacgacgttgtaaaacgc tga gga cct ggt cct ctg ac
US5P53/4R	agcggataacaatttcacacaggt tga agt ctc atg gaa gcc
P53/72	gcc aga ggc tgc tcc cc

[0284]

Masses				
Allele	Product Termination: ddC	SEQ #	Length	Mass
P53/72	gccagaggctgctcccc	5	17	5132.4
Cod72 G Arg (wt)	gccagaggctgctccccgc	6	19	5734.8
Cod72 C Pro	gccagaggctgctccccc	7	18	5405.6

[0285] Biotinylated US5 primer is used in the PCR amplification.

[0286] LPL-1 (N291S)

[0287] Amino acid exchange asparagine to serine at codon 291 of the lipoprotein lipase gene.

PCR Product length: 251 bp (SEQ ID NO: 15)

```
US4-LPL-F2                                                    (SEQ ID NO: 16)
gcgctccatt catctcttca tcgactctct gttgaatgaa gaaaatccaa gtaag-
gccta

caggtgcagt tccaaggaag cctttgagaa agggctctgc ttgagttgta gaaa-
gaaccg
            LPL-2                 291N

ctgcaacaatctgggctatgagatcaataa agtcagagcc aaaagaagca gcaaaat-
gta
                            g 291S

cctgaagact cgttctcaga tgccc
                US4-LPL-R2
```

[0288]

Primers (SEQ ID NOs: 16-18):	
US4-LPL-F2	cccagtcacgacgttgtaaaacgg cgc tcc att cat ctc ttc
US5-LPL-R2	agcggataacaatttcacacaggg ggc atc tga gaa cga gtc
LPL-2	caa tct ggg cta tga gat ca

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 118

<210> SEQ ID NO 1
<211> LENGTH: 361
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 1

ctgaggacct ggtcctctga ctgctctttt cacccatcta cagtccccct tgccgtccca 60

agcaatggat gatttgatgc tgtccccgga cgatattgaa caatggttca ctgaagaccc 120

aggtccagat gaagctccca gaatgccaga ggctgctccc cgcgtggccc ctgcaccagc 180

agctcctaca ccggcggccc ctgcaccagc cccctcctgg cccctgtcat cttctgtccc 240

ttcccagaaa acctaccagg gcagctacgg tttccgtctg ggcttcttgc attctgggac 300

agccaagtct gtgacttgca cggtcagttg ccctgagggg ctggcttcca tgagacttca 360

a 361

<210> SEQ ID NO 2
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 2

cccagtcacg acgttgtaaa acgctgagga cctggtcctc tgac 44

<210> SEQ ID NO 3
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 3

agcggataac aatttcacac aggttgaagt ctcatggaag cc 42

<210> SEQ ID NO 4
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 4

gccagaggct gctcccc 17

<210> SEQ ID NO 5
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 5

gccagaggct gctcccc 17

<210> SEQ ID NO 6

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<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 6

gccagaggct gctccccgc 19

<210> SEQ ID NO 7
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 7

gccagaggct gctccccc 18

<210> SEQ ID NO 8
<211> LENGTH: 161
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 8

gtccgtcaga acccatgcgg cagcaaggcc tgccgccgcc tcttcggccc agtggacagc 60

gagcagctga gccgcgactg tgatgcgcta atggcgggct gcatccagga ggcccgtgag 120

cgatggaact tcgactttgt caccgagaca ccactggagg g 161

<210> SEQ ID NO 9
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 9

cccagtcacg acgttgtaaa acggtccgtc agaacccatg cgg 43

<210> SEQ ID NO 10
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 10

agcggataac aatttcacac aggctccagt ggtgtctcgg tgac 44

<210> SEQ ID NO 11
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 11

cagcgagcag ctgag 15

<210> SEQ ID NO 12
<211> LENGTH: 15
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 12

cagcgagcag ctgag 15

<210> SEQ ID NO 13
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 13

cagcgagcag ctgagc 16

<210> SEQ ID NO 14
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 14

cagcgagcag ctgagac 17

<210> SEQ ID NO 15
<211> LENGTH: 205
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 15

gcgctccatt catctcttca tcgactctct gttgaatgaa gaaaatccaa gtaaggccta 60

caggtgcagt tccaaggaag cctttgagaa agggctctgc ttgagttgta gaaagaaccg 120

ctgcaacaat ctgggctatg agatcaataa agtcagagcc aaaagaagca gcaaaatgta 180

cctgaagact cgttctcaga tgccc 205

<210> SEQ ID NO 16
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primers

<400> SEQUENCE: 16

cccagtcacg acgttgtaaa acggcgctcc attcatctct tc 42

<210> SEQ ID NO 17
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 17

agcggataac aatttcacac agggggcatc tgagaacgag tc 42

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 18

caatctgggc tatgagatca 20

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 19

caatctgggc tatgagatca 20

<210> SEQ ID NO 20
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 20

caatctgggc tatgagatca a 21

<210> SEQ ID NO 21
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 21

caatctgggc tatgagatca gt 22

<210> SEQ ID NO 22
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 22

gtgccggcta ctcggatggc agcaaggact cctgcaaggg ggacagtgga ggcccacatg 60

<210> SEQ ID NO 23
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 23

ccacccacta ccggggcacg tggtacctga cgggcatcgt cagctggggc cagggctgcg 60

<210> SEQ ID NO 24
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 24

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cccagtcacg acgttgtaaa acgatggcag caaggactcc tg 42

<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 25

cacatgccac ccactacc 18

<210> SEQ ID NO 26
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 26

agcggataac aatttcacac aggtgacgat gccccgtcagg tac 43

<210> SEQ ID NO 27
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 27

atgccaccca ctacc 15

<210> SEQ ID NO 28
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 28

cacatgccac ccactaccg 19

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 29

cacatgccac ccactaccag 20

<210> SEQ ID NO 30
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Probe

<400> SEQUENCE: 30

agcggataac aatttcacac agg 23

<210> SEQ ID NO 31

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<211> LENGTH: 2363
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (138)...(2126)
<223> OTHER INFORMATION: AKAP-10
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank AF037439
<309> DATABASE ENTRY DATE: 1997-12-21

<400> SEQUENCE: 31

gcggcttgtt gataatatgg cggctggagc tgcctgggca tcccgaggag gcggtggggc     60

ccactcccgg aagaagggtc ccttttcgcg ctagtgcagc ggcccctctg gaccccggaag   120

tccgggccgg ttgctga atg agg gga gcc ggg ccc tcc ccg cgc cag tcc       170
                   Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser
                   1               5                   10

ccc cgc acc ctc cgt ccc gac ccg ggc ccc gcc atg tcc ttc ttc cgg      218
Pro Arg Thr Leu Arg Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg
            15                  20                  25

cgg aaa gtg aaa ggc aaa gaa caa gag aag acc tca gat gtg aag tcc      266
Arg Lys Val Lys Gly Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser
        30                  35                  40

att aaa gct tca ata tcc gta cat tcc cca caa aaa agc act aaa aat      314
Ile Lys Ala Ser Ile Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn
    45                  50                  55

cat gcc ttg ctg gag gct gca gga cca agt cat gtt gca atc aat gcc      362
His Ala Leu Leu Glu Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala
60                  65                  70                  75

att tct gcc aac atg gac tcc ttt tca agt agc agg aca gcc aca ctt      410
Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu
                80                  85                  90

aag aag cag cca agc cac atg gag gct gct cat ttt ggt gac ctg ggc      458
Lys Lys Gln Pro Ser His Met Glu Ala Ala His Phe Gly Asp Leu Gly
            95                  100                 105

aga tct tgt ctg gac tac cag act caa gag acc aaa tca agc ctt tct      506
Arg Ser Cys Leu Asp Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser
        110                 115                 120

aag acc ctt gaa caa gtc ttg cac gac act att gtc ctc cct tac ttc      554
Lys Thr Leu Glu Gln Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe
    125                 130                 135

att caa ttc atg gaa ctt cgg cga atg gag cat ttg gtg aaa ttt tgg      602
Ile Gln Phe Met Glu Leu Arg Arg Met Glu His Leu Val Lys Phe Trp
140                 145                 150                 155

tta gag gct gaa agt ttt cat tca aca act tgg tcg cga ata aga gca      650
Leu Glu Ala Glu Ser Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala
                160                 165                 170

cac agt cta aac aca atg aag cag agc tca ctg gct gag cct gtc tct      698
His Ser Leu Asn Thr Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser
            175                 180                 185

cca tct aaa aag cat gaa act aca gcg tct ttt tta act gat tct ctt      746
Pro Ser Lys Lys His Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu
        190                 195                 200

gat aag aga ttg gag gat tct ggc tca gca cag ttg ttt atg act cat      794
Asp Lys Arg Leu Glu Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His
    205                 210                 215

tca gaa gga att gac ctg aat aat aga act aac agc act cag aat cac      842
Ser Glu Gly Ile Asp Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His
220                 225                 230                 235
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ttg ctg ctt tcc cag gaa tgt gac agt gcc cat tct ctc cgt ctt gaa        890
Leu Leu Leu Ser Gln Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu
            240                 245                 250

atg gcc aga gca gga act cac caa gtt tcc atg gaa acc caa gaa tct        938
Met Ala Arg Ala Gly Thr His Gln Val Ser Met Glu Thr Gln Glu Ser
        255                 260                 265

tcc tct aca ctt aca gta gcc agt aga aat agt ccc gct tct cca cta        986
Ser Ser Thr Leu Thr Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu
    270                 275                 280

aaa gaa ttg tca gga aaa cta atg aaa agt ata gaa caa gat gca gtg       1034
Lys Glu Leu Ser Gly Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val
    285                 290                 295

aat act ttt acc aaa tat ata tct cca gat gct gct aaa cca ata cca       1082
Asn Thr Phe Thr Lys Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro
300                 305                 310                 315

att aca gaa gca atg aga aat gac atc ata gca agg att tgt gga gaa       1130
Ile Thr Glu Ala Met Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu
                320                 325                 330

gat gga cag gtg gat ccc aac tgt ttc gtt ttg gca cag tcc ata gtc       1178
Asp Gly Gln Val Asp Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val
            335                 340                 345

ttt agt gca atg gag caa gag cac ttt agt gag ttt ctg cga agt cac       1226
Phe Ser Ala Met Glu Gln Glu His Phe Ser Glu Phe Leu Arg Ser His
        350                 355                 360

cat ttc tgt aaa tac cag att gaa gtg ctg acc agt gga act gtt tac       1274
His Phe Cys Lys Tyr Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr
    365                 370                 375

ctg gct gac att ctc ttc tgt gag tca gcc ctc ttt tat ttc tct gag       1322
Leu Ala Asp Ile Leu Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu
380                 385                 390                 395

tac atg gaa aaa gag gat gca gtg aat atc tta caa ttc tgg ttg gca       1370
Tyr Met Glu Lys Glu Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala
                400                 405                 410

gca gat aac ttc cag tct cag ctt gct gcc aaa aag ggg caa tat gat       1418
Ala Asp Asn Phe Gln Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp
            415                 420                 425

gga cag gag gca cag aat gat gcc atg att tta tat gac aag tac ttc       1466
Gly Gln Glu Ala Gln Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe
        430                 435                 440

tcc ctc caa gcc aca cat cct ctt gga ttt gat gat gtt gta cga tta       1514
Ser Leu Gln Ala Thr His Pro Leu Gly Phe Asp Asp Val Val Arg Leu
    445                 450                 455

gaa att gaa tcc aat atc tgc agg gaa ggt ggg cca ctc ccc aac tgt       1562
Glu Ile Glu Ser Asn Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys
460                 465                 470                 475

ttc aca act cca tta cgt cag gcc tgg aca acc atg gag aag gtc ttt       1610
Phe Thr Thr Pro Leu Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe
                480                 485                 490

ttg cct ggc ttt ctg tcc agc aat ctt tat tat aaa tat ttg aat gat       1658
Leu Pro Gly Phe Leu Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp
            495                 500                 505

ctc atc cat tcg gtt cga gga gat gaa ttt ctg ggc ggg aac gtg tcg       1706
Leu Ile His Ser Val Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser
        510                 515                 520

ccg act gct cct ggc tct gtt ggc cct cct gat gag tct cac cca ggg       1754
Pro Thr Ala Pro Gly Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly
    525                 530                 535
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```
agt tct gac agc tct gcg tct cag tcc agt gtg aaa aaa gcc agt att      1802
Ser Ser Asp Ser Ser Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile
540                 545                 550                 555

aaa ata ctg aaa aat ttt gat gaa gcg ata att gtg gat gcg gca agt      1850
Lys Ile Leu Lys Asn Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser
                560                 565                 570

ctg gat cca gaa tct tta tat caa cgg aca tat gcc ggg aag atg aca      1898
Leu Asp Pro Glu Ser Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr
            575                 580                 585

ttt gga aga gtg agt gac ttg ggg caa ttc atc cgg gaa tct gag cct      1946
Phe Gly Arg Val Ser Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro
        590                 595                 600

gaa cct gat gta agg aaa tca aaa gga tcc atg ttc tca caa gct atg      1994
Glu Pro Asp Val Arg Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met
    605                 610                 615

aag aaa tgg gtg caa gga aat act gat gag gcc cag gaa gag cta gct      2042
Lys Lys Trp Val Gln Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala
620                 625                 630                 635

tgg aag att gct aaa atg ata gtc agt gac att atg cag cag gct cag      2090
Trp Lys Ile Ala Lys Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln
                640                 645                 650

tat gat caa ccg tta gag aaa tct aca aag tta tga ctcaaaactt           2136
Tyr Asp Gln Pro Leu Glu Lys Ser Thr Lys Leu  *
            655                 660

gagataaagg aaatctgctt gtgaaaaata agagaacttt tttcccttgg ttggattctt    2196

caacacagcc aatgaaaaca gcactatatt tctgatctgt cactgttgtt tccagggaga    2256

gaatggggag acaatcctag gacttccacc ctaatgcagt tacctgtagg gcataattgg    2316

atggcacatg atgtttcaca cagtgaggag tctttaaagg ttaccaa                 2363


<210> SEQ ID NO 32
<211> LENGTH: 662
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 32

Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser Pro Arg Thr Leu Arg
 1               5                  10                  15

Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg Arg Lys Val Lys Gly
            20                  25                  30

Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser Ile Lys Ala Ser Ile
        35                  40                  45

Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn His Ala Leu Leu Glu
    50                  55                  60

Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala Ile Ser Ala Asn Met
65                  70                  75                  80

Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu Lys Lys Gln Pro Ser
                85                  90                  95

His Met Glu Ala Ala His Phe Gly Asp Leu Gly Arg Ser Cys Leu Asp
            100                 105                 110

Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser Lys Thr Leu Glu Gln
        115                 120                 125

Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe Ile Gln Phe Met Glu
    130                 135                 140
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Leu Arg Arg Met Glu His Leu Val Lys Phe Trp Leu Glu Ala Glu Ser
145                 150                 155                 160

Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala His Ser Leu Asn Thr
                165                 170                 175

Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser Pro Ser Lys Lys His
            180                 185                 190

Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu Asp Lys Arg Leu Glu
        195                 200                 205

Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His Ser Glu Gly Ile Asp
    210                 215                 220

Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His Leu Leu Leu Ser Gln
225                 230                 235                 240

Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu Met Ala Arg Ala Gly
                245                 250                 255

Thr His Gln Val Ser Met Glu Thr Gln Glu Ser Ser Ser Thr Leu Thr
            260                 265                 270

Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu Lys Glu Leu Ser Gly
        275                 280                 285

Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val Asn Thr Phe Thr Lys
    290                 295                 300

Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro Ile Thr Glu Ala Met
305                 310                 315                 320

Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu Asp Gly Gln Val Asp
                325                 330                 335

Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val Phe Ser Ala Met Glu
            340                 345                 350

Gln Glu His Phe Ser Glu Phe Leu Arg Ser His His Phe Cys Lys Tyr
        355                 360                 365

Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr Leu Ala Asp Ile Leu
    370                 375                 380

Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu Tyr Met Glu Lys Glu
385                 390                 395                 400

Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala Ala Asp Asn Phe Gln
                405                 410                 415

Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp Gly Gln Glu Ala Gln
            420                 425                 430

Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe Ser Leu Gln Ala Thr
        435                 440                 445

His Pro Leu Gly Phe Asp Asp Val Val Arg Leu Glu Ile Glu Ser Asn
    450                 455                 460

Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys Phe Thr Thr Pro Leu
465                 470                 475                 480

Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe Leu Pro Gly Phe Leu
                485                 490                 495

Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp Leu Ile His Ser Val
            500                 505                 510

Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser Pro Thr Ala Pro Gly
        515                 520                 525

Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly Ser Ser Asp Ser Ser
    530                 535                 540

Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile Lys Ile Leu Lys Asn
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545                 550                 555                 560

Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser Leu Asp Pro Glu Ser
                565                 570                 575

Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr Phe Gly Arg Val Ser
            580                 585                 590

Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro Glu Pro Asp Val Arg
        595                 600                 605

Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met Lys Lys Trp Val Gln
    610                 615                 620

Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala Trp Lys Ile Ala Lys
625                 630                 635                 640

Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln Tyr Asp Gln Pro Leu
                645                 650                 655

Glu Lys Ser Thr Lys Leu
            660


<210> SEQ ID NO 33
<211> LENGTH: 2363
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (138)...(2126)
<223> OTHER INFORMATION: AKAP-10-5
<220> FEATURE:
<221> NAME/KEY: allele
<222> LOCATION: 2073
<223> OTHER INFORMATION: Single Nucleotide Polymorphism: A to G

<400> SEQUENCE: 33

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tccgggccgg ttgctga atg agg gga gcc ggg ccc tcc ccg cgc cag tcc        170
                   Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser
                   1               5                   10

ccc cgc acc ctc cgt ccc gac ccg ggc ccc gcc atg tcc ttc ttc cgg        218
Pro Arg Thr Leu Arg Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg
            15                  20                  25

cgg aaa gtg aaa ggc aaa gaa caa gag aag acc tca gat gtg aag tcc        266
Arg Lys Val Lys Gly Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser
        30                  35                  40

att aaa gct tca ata tcc gta cat tcc cca caa aaa agc act aaa aat        314
Ile Lys Ala Ser Ile Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn
    45                  50                  55

cat gcc ttg ctg gag gct gca gga cca agt cat gtt gca atc aat gcc        362
His Ala Leu Leu Glu Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala
60                  65                  70                  75

att tct gcc aac atg gac tcc ttt tca agt agc agg aca gcc aca ctt        410
Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu
                80                  85                  90

aag aag cag cca agc cac atg gag gct gct cat ttt ggt gac ctg ggc        458
Lys Lys Gln Pro Ser His Met Glu Ala Ala His Phe Gly Asp Leu Gly
            95                  100                 105

aga tct tgt ctg gac tac cag act caa gag acc aaa tca agc ctt tct        506
Arg Ser Cys Leu Asp Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser
        110                 115                 120

aag acc ctt gaa caa gtc ttg cac gac act att gtc ctc cct tac ttc        554
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Lys Thr Leu Glu Gln Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe
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att caa ttc atg gaa ctt cgg cga atg gag cat ttg gtg aaa ttt tgg      602
Ile Gln Phe Met Glu Leu Arg Arg Met Glu His Leu Val Lys Phe Trp
140                 145                 150                 155

tta gag gct gaa agt ttt cat tca aca act tgg tcg cga ata aga gca      650
Leu Glu Ala Glu Ser Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala
                160                 165                 170

cac agt cta aac aca atg aag cag agc tca ctg gct gag cct gtc tct      698
His Ser Leu Asn Thr Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser
            175                 180                 185

cca tct aaa aag cat gaa act aca gcg tct ttt tta act gat tct ctt      746
Pro Ser Lys Lys His Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu
        190                 195                 200

gat aag aga ttg gag gat tct ggc tca gca cag ttg ttt atg act cat      794
Asp Lys Arg Leu Glu Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His
    205                 210                 215

tca gaa gga att gac ctg aat aat aga act aac agc act cag aat cac      842
Ser Glu Gly Ile Asp Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His
220                 225                 230                 235

ttg ctg ctt tcc cag gaa tgt gac agt gcc cat tct ctc cgt ctt gaa      890
Leu Leu Leu Ser Gln Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu
                240                 245                 250

atg gcc aga gca gga act cac caa gtt tcc atg gaa acc caa gaa tct      938
Met Ala Arg Ala Gly Thr His Gln Val Ser Met Glu Thr Gln Glu Ser
            255                 260                 265

tcc tct aca ctt aca gta gcc agt aga aat agt ccc gct tct cca cta      986
Ser Ser Thr Leu Thr Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu
        270                 275                 280

aaa gaa ttg tca gga aaa cta atg aaa agt ata gaa caa gat gca gtg     1034
Lys Glu Leu Ser Gly Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val
    285                 290                 295

aat act ttt acc aaa tat ata tct cca gat gct gct aaa cca ata cca     1082
Asn Thr Phe Thr Lys Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro
300                 305                 310                 315

att aca gaa gca atg aga aat gac atc ata gca agg att tgt gga gaa     1130
Ile Thr Glu Ala Met Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu
                320                 325                 330

gat gga cag gtg gat ccc aac tgt ttc gtt ttg gca cag tcc ata gtc     1178
Asp Gly Gln Val Asp Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val
            335                 340                 345

ttt agt gca atg gag caa gag cac ttt agt gag ttt ctg cga agt cac     1226
Phe Ser Ala Met Glu Gln Glu His Phe Ser Glu Phe Leu Arg Ser His
        350                 355                 360

cat ttc tgt aaa tac cag att gaa gtg ctg acc agt gga act gtt tac     1274
His Phe Cys Lys Tyr Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr
    365                 370                 375

ctg gct gac att ctc ttc tgt gag tca gcc ctc ttt tat ttc tct gag     1322
Leu Ala Asp Ile Leu Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu
380                 385                 390                 395

tac atg gaa aaa gag gat gca gtg aat atc tta caa ttc tgg ttg gca     1370
Tyr Met Glu Lys Glu Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala
                400                 405                 410

gca gat aac ttc cag tct cag ctt gct gcc aaa aag ggg caa tat gat     1418
Ala Asp Asn Phe Gln Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp
            415                 420                 425

gga cag gag gca cag aat gat gcc atg att tta tat gac aag tac ttc     1466
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Gly Gln Glu Ala Gln Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe
        430                 435                 440

tcc ctc caa gcc aca cat cct ctt gga ttt gat gat gtt gta cga tta      1514
Ser Leu Gln Ala Thr His Pro Leu Gly Phe Asp Asp Val Val Arg Leu
    445                 450                 455

gaa att gaa tcc aat atc tgc agg gaa ggt ggg cca ctc ccc aac tgt      1562
Glu Ile Glu Ser Asn Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys
460                 465                 470                 475

ttc aca act cca tta cgt cag gcc tgg aca acc atg gag aag gtc ttt      1610
Phe Thr Thr Pro Leu Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe
                480                 485                 490

ttg cct ggc ttt ctg tcc agc aat ctt tat tat aaa tat ttg aat gat      1658
Leu Pro Gly Phe Leu Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp
            495                 500                 505

ctc atc cat tcg gtt cga gga gat gaa ttt ctg ggc ggg aac gtg tcg      1706
Leu Ile His Ser Val Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser
        510                 515                 520

ccg act gct cct ggc tct gtt ggc cct cct gat gag tct cac cca ggg      1754
Pro Thr Ala Pro Gly Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly
    525                 530                 535

agt tct gac agc tct gcg tct cag tcc agt gtg aaa aaa gcc agt att      1802
Ser Ser Asp Ser Ser Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile
540                 545                 550                 555

aaa ata ctg aaa aat ttt gat gaa gcg ata att gtg gat gcg gca agt      1850
Lys Ile Leu Lys Asn Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser
                560                 565                 570

ctg gat cca gaa tct tta tat caa cgg aca tat gcc ggg aag atg aca      1898
Leu Asp Pro Glu Ser Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr
            575                 580                 585

ttt gga aga gtg agt gac ttg ggg caa ttc atc cgg gaa tct gag cct      1946
Phe Gly Arg Val Ser Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro
        590                 595                 600

gaa cct gat gta agg aaa tca aaa gga tcc atg ttc tca caa gct atg      1994
Glu Pro Asp Val Arg Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met
    605                 610                 615

aag aaa tgg gtg caa gga aat act gat gag gcc cag gaa gag cta gct      2042
Lys Lys Trp Val Gln Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala
620                 625                 630                 635

tgg aag att gct aaa atg ata gtc agt gac gtt atg cag cag gct cag      2090
Trp Lys Ile Ala Lys Met Ile Val Ser Asp Val Met Gln Gln Ala Gln
                640                 645                 650

tat gat caa ccg tta gag aaa tct aca aag tta tga ctcaaaactt           2136
Tyr Asp Gln Pro Leu Glu Lys Ser Thr Lys Leu  *
            655                 660

gagataaagg aaatctgctt gtgaaaaata agagaacttt tttcccttgg ttggattctt    2196

caacacagcc aatgaaaaca gcactatatt tctgatctgt cactgttgtt tccagggaga    2256

gaatggggag acaatcctag gacttccacc ctaatgcagt tacctgtagg gcataattgg    2316

atggcacatg atgtttcaca cagtgaggag tctttaaagg ttaccaa                  2363


<210> SEQ ID NO 34
<211> LENGTH: 662
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 34

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Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg Arg Lys Val Lys Gly
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Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser Ile Lys Ala Ser Ile
        35                  40                  45

Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn His Ala Leu Leu Glu
    50                  55                  60

Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala Ile Ser Ala Asn Met
65                  70                  75                  80

Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu Lys Lys Gln Pro Ser
                85                  90                  95

His Met Glu Ala Ala His Phe Gly Asp Leu Gly Arg Ser Cys Leu Asp
            100                 105                 110

Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser Lys Thr Leu Glu Gln
        115                 120                 125

Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe Ile Gln Phe Met Glu
    130                 135                 140

Leu Arg Arg Met Glu His Leu Val Lys Phe Trp Leu Glu Ala Glu Ser
145                 150                 155                 160

Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala His Ser Leu Asn Thr
                165                 170                 175

Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser Pro Ser Lys Lys His
            180                 185                 190

Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu Asp Lys Arg Leu Glu
        195                 200                 205

Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His Ser Glu Gly Ile Asp
    210                 215                 220

Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His Leu Leu Leu Ser Gln
225                 230                 235                 240

Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu Met Ala Arg Ala Gly
                245                 250                 255

Thr His Gln Val Ser Met Glu Thr Gln Glu Ser Ser Ser Thr Leu Thr
            260                 265                 270

Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu Lys Glu Leu Ser Gly
        275                 280                 285

Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val Asn Thr Phe Thr Lys
    290                 295                 300

Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro Ile Thr Glu Ala Met
305                 310                 315                 320

Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu Asp Gly Gln Val Asp
                325                 330                 335

Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val Phe Ser Ala Met Glu
            340                 345                 350

Gln Glu His Phe Ser Glu Phe Leu Arg Ser His His Phe Cys Lys Tyr
        355                 360                 365

Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr Leu Ala Asp Ile Leu
    370                 375                 380

Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu Tyr Met Glu Lys Glu
385                 390                 395                 400

Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala Ala Asp Asn Phe Gln
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Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp Gly Gln Glu Ala Gln
420 425 430

Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe Ser Leu Gln Ala Thr
435 440 445

His Pro Leu Gly Phe Asp Asp Val Val Arg Leu Glu Ile Glu Ser Asn
450 455 460

Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys Phe Thr Thr Pro Leu
465 470 475 480

Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe Leu Pro Gly Phe Leu
485 490 495

Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp Leu Ile His Ser Val
500 505 510

Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser Pro Thr Ala Pro Gly
515 520 525

Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly Ser Ser Asp Ser Ser
530 535 540

Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile Lys Ile Leu Lys Asn
545 550 555 560

Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser Leu Asp Pro Glu Ser
565 570 575

Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr Phe Gly Arg Val Ser
580 585 590

Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro Glu Pro Asp Val Arg
595 600 605

Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met Lys Lys Trp Val Gln
610 615 620

Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala Trp Lys Ile Ala Lys
625 630 635 640

Met Ile Val Ser Asp Val Met Gln Gln Ala Gln Tyr Asp Gln Pro Leu
645 650 655

Glu Lys Ser Thr Lys Leu
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<210> SEQ ID NO 35
<211> LENGTH: 162025
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank AC005730
<309> DATABASE ENTRY DATE: 1998-10-22

<400> SEQUENCE: 35

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<210> SEQ I NO 37
<211> LENGTH: 1350
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (213)...(920)
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank AJ242973
<309> DATABASE ENTRY DATE: 1999-10-26

<400> SEQUENCE: 37

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ccggccgcgg accccactct ctgccgttcc ggctgcggct ccgctgccgg tagcgccgtc     180

ccccgggacc acccttcggc tggcgccctc cc atg ctc tcg gcc acc cgg agg      233
                                    Met Leu Ser Ala Thr Arg Arg
                                     1               5

gct tgc cag ctc ctc ctc ctc cac agc ctc ttt ccc gtc ccg agg atg      281
Ala Cys Gln Leu Leu Leu Leu His Ser Leu Phe Pro Val Pro Arg Met
        10                  15                  20

ggc aac tcg gcc tcg aac atc gtc agc ccc cag gag gcc ttg ccg ggc      329
Gly Asn Ser Ala Ser Asn Ile Val Ser Pro Gln Glu Ala Leu Pro Gly
    25                  30                  35

cgg aag gaa cag acc cct gta gcg gcc aaa cat cat gtc aat ggc aac      377
Arg Lys Glu Gln Thr Pro Val Ala Ala Lys His His Val Asn Gly Asn
 40                  45                  50                  55

aga aca gtc gaa cct ttc cca gag gga aca cag atg gct gta ttt gga      425
Arg Thr Val Glu Pro Phe Pro Glu Gly Thr Gln Met Ala Val Phe Gly
                 60                  65                  70

atg gga tgt ttc tgg gga gct gaa agg aaa ttc tgg gtc ttg aaa gga      473
Met Gly Cys Phe Trp Gly Ala Glu Arg Lys Phe Trp Val Leu Lys Gly
             75                  80                  85

gtg tat tca act caa gtt ggt ttt gca gga ggc tat act tca aat cct      521
Val Tyr Ser Thr Gln Val Gly Phe Ala Gly Gly Tyr Thr Ser Asn Pro
         90                  95                 100

act tat aaa gaa gtc tgc tca gaa aaa act ggc cat gca gaa gtc gtc      569
Thr Tyr Lys Glu Val Cys Ser Glu Lys Thr Gly His Ala Glu Val Val
    105                 110                 115

cga gtg gtg tac cag cca gaa cac atg agt ttt gag gaa ctg ctc aag      617
Arg Val Val Tyr Gln Pro Glu His Met Ser Phe Glu Glu Leu Leu Lys
120                 125                 130                 135

gtc ttc tgg gag aat cac gac ccg acc caa ggt atg cgc cag ggg aac      665
Val Phe Trp Glu Asn His Asp Pro Thr Gln Gly Met Arg Gln Gly Asn
                140                 145                 150

gac cat ggc act cag tac cgc tcg gcc atc tac ccg acc tct gcc aag      713
Asp His Gly Thr Gln Tyr Arg Ser Ala Ile Tyr Pro Thr Ser Ala Lys
            155                 160                 165

caa atg gag gca gcc ctg agc tcc aaa gag aac tac caa aag gtt ctt      761
Gln Met Glu Ala Ala Leu Ser Ser Lys Glu Asn Tyr Gln Lys Val Leu
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tca gag cac ggc ttc ggc ccc atc act acc gac atc cgg gag gga cag       809
Ser Glu His Gly Phe Gly Pro Ile Thr Thr Asp Ile Arg Glu Gly Gln
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act ttc tac tat gcg gaa gac tac cac cag cag tac ctg agc aag aac       857
Thr Phe Tyr Tyr Ala Glu Asp Tyr His Gln Gln Tyr Leu Ser Lys Asn
200                 205                 210                 215

ccc aat ggc tac tgc ggc ctt ggg ggc acc ggc gtg tcc tgc cca gtg       905
Pro Asn Gly Tyr Cys Gly Leu Gly Gly Thr Gly Val Ser Cys Pro Val
                220                 225                 230

ggt att aaa aaa taa ttgctcccca catggtgggc ctttgaggtt ccagtaaaaa       960
Gly Ile Lys Lys  *
            235

tgctttcaac aaattgggca atgcttgtgt gattcacaat cgtggcattt aaagtgcaca    1020

aagtacaaag gaatttatac agattgggtt taccgaagta taatctatag gaggcgcgat    1080

ggcaagttga taaaatgtga cttatctcct aataagttat ggtgggagtg gagctgtgcg    1140

gtttcctgtg tcttctgggg tctgagtgaa gatagcaggg atgctgtgtt caccccttctt   1200

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<210> SEQ ID NO 38
<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: Homo Sapien

<400> SEQUENCE: 38

Met Leu Ser Ala Thr Arg Arg Ala Cys Gln Leu Leu Leu Leu His Ser
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Leu Phe Pro Val Pro Arg Met Gly Asn Ser Ala Ser Asn Ile Val Ser
            20                  25                  30

Pro Gln Glu Ala Leu Pro Gly Arg Lys Glu Gln Thr Pro Val Ala Ala
        35                  40                  45

Lys His His Val Asn Gly Asn Arg Thr Val Glu Pro Phe Pro Glu Gly
    50                  55                  60

Thr Gln Met Ala Val Phe Gly Met Gly Cys Phe Trp Gly Ala Glu Arg
65                  70                  75                  80

Lys Phe Trp Val Leu Lys Gly Val Tyr Ser Thr Gln Val Gly Phe Ala
                85                  90                  95

Gly Gly Tyr Thr Ser Asn Pro Thr Tyr Lys Glu Val Cys Ser Glu Lys
            100                 105                 110

Thr Gly His Ala Glu Val Val Arg Val Val Tyr Gln Pro Glu His Met
        115                 120                 125

Ser Phe Glu Glu Leu Leu Lys Val Phe Trp Glu Asn His Asp Pro Thr
    130                 135                 140

Gln Gly Met Arg Gln Gly Asn Asp His Gly Thr Gln Tyr Arg Ser Ala
145                 150                 155                 160

Ile Tyr Pro Thr Ser Ala Lys Gln Met Glu Ala Ala Leu Ser Ser Lys
                165                 170                 175

Glu Asn Tyr Gln Lys Val Leu Ser Glu His Gly Phe Gly Pro Ile Thr
            180                 185                 190

Thr Asp Ile Arg Glu Gly Gln Thr Phe Tyr Tyr Ala Glu Asp Tyr His
        195                 200                 205
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Gln Gln Tyr Leu Ser Lys Asn Pro Asn Gly Tyr Cys Gly Leu Gly Gly
    210                 215                 220

Thr Gly Val Ser Cys Pro Val Gly Ile Lys Lys
225                 230                 235
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<210> SEQ ID NO 39
<211> LENGTH: 481
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank AW195104
<309> DATABASE ENTRY DATE: 1999-11-29

<400> SEQUENCE: 39

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ctgaggctat acacgtttgc aaattgtaag gcactggcgg gcagagagca cagataaagg    240

acttctgggg tcccccatcc tgtccagcaa cctcccagct cacaccttag cttctaccaa    300

gaagggtgaa cacagcatcc ctgctatctt cactcagacc ccagaaaacc cagggaaacc    360

cgacagctcc actcccacca taacttatta ggagataagt cacattttat caacttgcca    420

tcgcgcctcc tatagattat acttcggtaa acccaatctg tataaattcc tttgtacttt    480

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<210> SEQ ID NO 40
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Homo Sapien
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: GenBank AW874187
<309> DATABASE ENTRY DATE: 2000-05-22

<400> SEQUENCE: 40

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agagaaagcc ccttatgtat gtctagattt ggtaaagcga aggatttcag cgaatgagtc    180

actgaggcta tacacgtttg caaattgtaa ggcactggcg ggcagagagc acagataaag    240

gacttttggg ggtcccccat tcctgtccag caacctccca gctcacacct tagcttctac    300

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aaccgcacag ctccactccc accataactt                                     390
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<210> SEQ ID NO 41
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 41

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<210> SEQ ID NO 42
<211> LENGTH: 22

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 42

gtccaatata tgcaaacagt tg 22

<210> SEQ ID NO 43
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 43

agcggataac aatttcacac agg 23

<210> SEQ ID NO 44
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 44

actgagcctg ctgcataa 18

<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 45

tctcaatcat gtgcattgag g 21

<210> SEQ ID NO 46
<211> LENGTH: 43
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 46

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<210> SEQ ID NO 47
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: oligonucleotide primer

<400> SEQUENCE: 47

agcggataac aatttcacac agg 23

<210> SEQ ID NO 48
<211> LENGTH: 18
<212> TYPE: DNA
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<400> SEQUENCE: 48

ctggcgccac gtggtcaa 18

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<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 49

tttctctgca cagagagggc 20

<210> SEQ ID NO 50
<211> LENGTH: 44
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 50

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<210> SEQ ID NO 51
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 51

agcggataac aatttcacac agg 23

<210> SEQ ID NO 52
<211> LENGTH: 18
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 52

ctgaaaaggg agagaaag 18

<210> SEQ ID NO 53
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 53

tcccaaagtg ctggaattac 20

<210> SEQ ID NO 54
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Primer

<400> SEQUENCE: 54

gtccaatata tgcaaacagt tg 22

<210> SEQ ID NO 55
<211> LENGTH: 20

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<212> TYPE: DNA
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<400> SEQUENCE: 55

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<210> SEQ ID NO 56
<211> LENGTH: 18
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 56

gcgctcctgt cggtgcca 18

<210> SEQ ID NO 57
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 57

gcctgactgg tggggccc 18

<210> SEQ ID NO 58
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 58

catgcatgca cggtc 15

<210> SEQ ID NO 59
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 59

cagagagtac ccctcgaccg tgcatgcatg 30

<210> SEQ ID NO 60
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 60

catgcatgca cggtt 15

<210> SEQ ID NO 61
<211> LENGTH: 30
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 61

gtacgtacgt gccaactccc catgagagac 30

<210> SEQ ID NO 62
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 62

catgcatgca cggt 14

<210> SEQ ID NO 63
<211> LENGTH: 18
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 63

gcctgactgg tggggccc 18

<210> SEQ ID NO 64
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 64

gtgctgcagg tgtaaacttg taccag 26

<210> SEQ ID NO 65
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 65

cacggatccg gtagcagcgg tagagttg 28

<210> SEQ ID NO 66
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 66

actgggcatg tggagacag 19

<210> SEQ ID NO 67
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 67

gcactttctt gccatgag 18

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<210> SEQ ID NO 68
<211> LENGTH: 14
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 68

tcagtcacga cgtt 14

<210> SEQ ID NO 69
<211> LENGTH: 14
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 69

cggataacaa tttc 14

<210> SEQ ID NO 70
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 70

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<210> SEQ ID NO 71
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 71

caatttcaca cagcggatgc ttcttttggc tctgact 37

<210> SEQ ID NO 72
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 72

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<210> SEQ ID NO 73
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 73

cggataacaa tttcggatgc actgggagca ttgaggc 37

<210> SEQ ID NO 74
<211> LENGTH: 38

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 74

tcagtcacga cgttggatga gcagatccct ggacaggc 38

<210> SEQ ID NO 75
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 75

cggataacaa tttcggatgg acaaaatacc tgtattcc 38

<210> SEQ ID NO 76
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 76

tcagtcacga cgttggatgc agagcagctc cgagtc 36

<210> SEQ ID NO 77
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 77

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<210> SEQ ID NO 78
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 78

tcagtcacga cgttggatgc ccacatgcca cccactac 38

<210> SEQ ID NO 79
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 79

cggataacaa tttcggatgc ccgtcaggta ccacg 35

<210> SEQ ID NO 80
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

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<400> SEQUENCE: 80

tcagtcacga cgttggatgc ccacagtgga gcttcag 37

<210> SEQ ID NO 81
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 81

gctcatacct tgcaggatga cg 22

<210> SEQ ID NO 82
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 82

tcagtcacga cgttggatga ccagctgttc gtgttc 36

<210> SEQ ID NO 83
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 83

tacatggagt tcggggatgc acacggcgac tctc 34

<210> SEQ ID NO 84
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 84

tcagtcacga cgttggatgg ggaagagcag agatatacgt 40

<210> SEQ ID NO 85
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 85

gaggggctga tccaggatgg gtgctccac 29

<210> SEQ ID NO 86
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 86

tgaagcactt gaaggatgag ggtgtctgcg 30

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<210> SEQ ID NO 87
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 87

cggataacaa tttcggatgc tgcgtgatga tgaaatcg 38

<210> SEQ ID NO 88
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 88

gatgaagctc ccaggatgcc agaggc 26

<210> SEQ ID NO 89
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 89

gccgccggtg taggatgctg ctggtgc 27

<210> SEQ ID NO 90
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide Template

<400> SEQUENCE: 90

cgcagggttt cctcgtcgca ctgggcatgt g 31

<210> SEQ ID NO 91
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Biotinylatd primer

<400> SEQUENCE: 91

tgcttatccc tgtagctacc ctgtcttggc cttgcagatc caa 43

<210> SEQ ID NO 92
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 92

agcggataac aatttcacac aggccatcac accgcggtac tg 42

<210> SEQ ID NO 93
<211> LENGTH: 44

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 93

cccagtcacg acgttgtaaa acgtcttggc cttgcagatc caag 44

<210> SEQ ID NO 94
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 94

agcggataac aatttcacac aggccatcac accgcggtac tg 42

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 95

ctccagctgg gcaggagtgc 20

<210> SEQ ID NO 96
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer

<400> SEQUENCE: 96

cacttcagtc gctccct 17

<210> SEQ ID NO 97
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Biotinylated primer

<400> SEQUENCE: 97

cccagtcacg acgttgtaaa acg 23

<210> SEQ ID NO 98
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 98

cctttgagaa agggctctgc ttgagttgta gaaagaaccg ctgcaacaat ctgggctatg 60

agatcaataa agtcagagcc aaaagaagca gcaaaatgta 100

<210> SEQ ID NO 99
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 99

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cctttgagaa agggctctgc ttgagttgta gaaagaaccg ctgcaacaat ctgggctatg      60

agatcagtaa agtcagagcc aaaagaagca gcaaaatgta                            100


<210> SEQ ID NO 100
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 100

gaattatttt tgtgtttcta aaactatggt tcccaataaa agtgactctc agcgagcctc      60

aatgctccca gtgctattca tgggcagctc tctgggctca                            100


<210> SEQ ID NO 101
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 101

gaattatttt tgtgtttcta aaactatggt tcccaataaa agtgactctc agcaagcctc      60

aatgctccca gtgctattca tgggcagctc tctgggctca                            100


<210> SEQ ID NO 102
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 102

taataggact acttctaatc tgtaagagca gatccctgga caggcgagga atacaggtat      60

tttgtccttg aagtaacctt tcag                                             84


<210> SEQ ID NO 103
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 103

taataggact acttctaatc tgtaagagca gatccctgga caggcaagga atacaggtat      60

tttgtccttg aagtaacctt tcag                                             84


<210> SEQ ID NO 104
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 104

ctcaccatgg gcatttgatt gcagagcagc tccgagtccg tccagagctt cctgcagtca      60

atgatcaccg ctgtgggcat ccctgaggtc atgtctcgta                            100


<210> SEQ ID NO 105
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 105

ctcaccatgg gcatttgatt gcagagcagc tccgagtcca tccagagctt cctgcagtca      60

atgatcaccg ctgtgggcat ccctgaggtc atgtctcgta                            100
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<210> SEQ ID NO 106
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 106

agcaaggact cctgcaaggg ggacagtgga ggcccacatg ccacccacta ccagggcacg 60

tggtacctga cgggcatcgt cagctggggc cagggctgcg 100

<210> SEQ ID NO 107
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 107

agcaaggact cctgcaaggg ggacagtgga ggcccacatg ccacccacta ccggggcacg 60

tggtacctga cgggcatcgt cagctggggc cagggctgcg 100

<210> SEQ ID NO 108
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Hom sapien

<400> SEQUENCE: 108

caataactct aatgcagcgg aagatgacct gcccacagtg gagcttcagg gcgtggtgcc 60

ccggggcgtc aacctgcaag gtatgagcat acccccctc 100

<210> SEQ ID NO 109
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 109

caataactct aatgcagcgg aagatgacct gcccacagtg gagcttcagg gcttggtgcc 60

ccggggcgtc aacctgcaag gtatgagcat acccccctc 100

<210> SEQ ID NO 110
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 110

ttgaagcttt gggctacgtg gatgaccagc tgttcgtgtt ctatgatcat gagagtcgcc 60

gtgtggagcc ccgaactcca tgggtttcca gtagaatttc 100

<210> SEQ ID NO 111
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 111

ttgaagcttt gggctacgtg gatgaccagc tgttcgtgtt ctatgatgat gagagtcgcc 60

gtgtggagcc ccgaactcca tgggtttcca gtagaatttc 100

<210> SEQ ID NO 112
<211> LENGTH: 100

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<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 112

ggataacctt ggctgtaccc cctggggaag agcagagata tacgtgccag gtggagcacc 60

caggcctgga tcagcccctc attgtgatct gggagccctc 100

<210> SEQ ID NO 113
<211> LENGTH: 100
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 113

ggataacctt ggctgtaccc cctggggaag agcagagata tacgtaccag gtggagcacc 60

caggcctgga tcagcccctc attgtgatct gggagccctc 100

<210> SEQ ID NO 114
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 114

tgaagcactt gaaggagaag gtgtctgcgg gagccgattt catcatcacg cagcttttct 60

ttgaggctga cacattcttc 80

<210> SEQ ID NO 115
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 115

tgaagcactt gaaggagaag gtgtctgcgg gagtcgattt catcatcacg cagcttttct 60

ttgaggctga cacattcttc 80

<210> SEQ ID NO 116
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 116

tccagatgaa gctcccagaa tgccagaggc tgctccccgc gtggcccctg caccagcagc 60

tcctacaccg gcggcccctg 80

<210> SEQ ID NO 117
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Homo sapien

<400> SEQUENCE: 117

tccagatgaa gctcccagaa tgccagaggc tgctcccccc gtggcccctg caccagcagc 60

tcctacaccg gcggcccctg 80

<210> SEQ ID NO 118
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hair pin structure

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<400> SEQUENCE: 118

cagagagtac ccctcaaccg tgcatgcatg aaacatgcat gcacggtt                    48
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What is claimed is:

1. A method for determining whether a polymorphism correlates with suceptibility to morbidity, early mortality, or morbidity and early mortality, comprising;

identifying a polymorphism; and

determining the frequency of the polymorphism with increasing age in a healthy population.

2. The method of claim 1, wherein the polymorphism is identified by detecting the presence of target nucleic acids in a sample by a method, comprising the steps of:

a) hybridizing a first oligonucleotide to the target nucleic acid;

b) hybridizing a second oligonucleotide to an adjacent region of the target nucleic acid;

c) ligating the hybridized oligonucleotides; and

d) detecting hybridized first oligonucleotide by mass spectrometry as an indication of the presence of the target nucleic acid.

3. The method of claim 1, wherein the polymorphism is identified by detecting target nucleic acids in a sample by a method, comprising the steps of:

a) hybridizing a first oligonucleotide to the target nucleic acid and hybridizing a second oligonucleotide to an adjacent region of the target nucleic acid;

b) contacting the hybridized first and second oligonucleotides with a cleavage enzyme to form a cleavage product; and

c) detecting the cleavage product by mass spectrometry as an indication of the presence of the target nucleic acid.

4. The method of claim 3 wherein the samples are from subjects in a healthy database.

5. The method of claim 1, wherein the polymorphism is identified by identifying target nucleic acids in a sample by primer oligo base extension (probe).

6. The method of 5, wherein primer oligo base extension, comprises:

a) obtaining a nucleic acid molecule that contains a target nucleotide;

b) optionally immobilizing the nucleic acid molecule onto a solid support, to produce an immobilized nucleic acid molecule;

c) hybridizing the nucleic acid molecule with a primer oligonucleotide that is complementary to the nucleic acid molecule at a site adjacent to the target nucleotide;

d) contacting the product of step c) with a composition comprising a dideoxynucleoside triphosphate or a 3'-deoxynucleoside triphosphate and a polymerase, so that only a dideoxynucleoside or 3'-deoxynucleoside triphosphate that is complementary to the target nucleotide is extended onto the primer; and

e) detecting the extended primer, thereby identifying the target nucleotide.

7. The method of claim 6, wherein detection of the extended primer is effected by mass spectrometry, comprising:

ionizing and volatizing the product of step d) ; and

detecting the extended primer by mass spectrometry, thereby identifying the target nucleotide.

8. The method of claim 7, wherein;

samples are presented to the mass spectrometer as arrays on chips; and

each sample occupies a volume that is about the size of the laser spot projected by the laser in a mass spectrometer used in matrix-assisted laser desorption/ionization (MALDI) spectrometry.

9. A method for determining a polymorphism that correlates with age, ethnicity or gender, comprising:

identifying a polymorphism; and

determining the frequency of the polymorphism with increasing age, with ethnicity or with gender in a healthy population.

10. The method of claim 1, wherein the polymorphism is identified by a method, comprising:

sorting the database according to a selected parameter to identify samples that match the selected parameter, wherein the database comprises:

datapoints representative of a plurality of healthy organisms from whom biological samples are obtained, wherein each

datapoint is associated with data representative of the organism type and other identifying information.;

isolating a biopolymer from each identified sample;

optionally pooling each isolated biopolymer;

optionally amplifying the amount of biopolymer;

cleaving the pooled biopolymers to produce fragments thereof;

obtaining a mass spectrum of the resulting fragments and comparing the mass spectrum with a control mass spectrum to identify differences between the spectra and thereby identifing any polymorphisms; wherein:

the control mass spectrum is obtained from unsorted samples in the collection or samples sorted according to a different parameter.

11. The method of claim 10, wherein cleaving is effected by contacting the biopolymer with an enzyme.

12. The method of claim 11, wherein the enzyme is selected from the group consisting of nucleotide glycosylase, a nickase and a type IIS restriction enzyme.

13. The method of claim 10, wherein the biopolymer is a nucleic acid or a protein.

14. The method of claim 10, wherein the the mass spectrometric format is selected from among Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI, Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof.

15. The method of claim 1, wherein the polymorphism is identified by a method, comprising:

obtaining samples of body tissue or fluid from a plurality of organisms;

isolating a biopolymer from each sample;

pooling each isolated biopolymer;

optionally amplifying the amount of biopolymer;

cleaving the pooled biopolymers to produce fragments thereof;

obtaining a mass spectrum of the resulting fragments;

comparing the frequency of each fragment to identify fragments present in amounts lower than the average frequency, thereby identifying any polymorphisms.

16. The method of claim 15, wherein cleaving is effected by contacting the biopolymer with an enzyme.

17. The method of claim 16, wherein the enzyme is selected from the group consisting of nucleotide glycosylase, a nickase and a type IIS restriction enzyme.

18. The method of claim 15, wherein the biopolymer is a nucleic acid or a protein.

19. The method of claim 15, wherein the the mass spectrometric format is selected from among Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI, Ion Cyclotron Resonance (ICR), Fourier Transform and combinations thereof.

20. The method of claim 15, wherein the samples are obtained from healthy subjects.

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